Modelling, analysis and control of biological networks

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Modélisation, analyse et commande des réseaux biologiques

Thèse dirigée par Jean Luc GOUZÉ (Inria).

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Abstract

The purpose of this thesis is the modeling, reduction, analysis and control of biological systems. Modeling of biological networks is done by differential equations; the systems are typically nonlinear, of large dimensions, with different time scales, and complex to analyze. First, using techniques of monotone and compartmental systems, we study the global stability of the equilibrium of Michaelis-Menten enzymatic model without any approximation, when the system is closed or opened; we also study the general case of a chain of enzymatic reactions. Biological networks are generally composed of two parts in interaction (genetic and metabolic), we therefore investigate different types of models coupling metabolic reactions chains with a genetic system; we reduce the full system based on the difference in time scales (Tikhonov theorem). In the second part, we apply the same techniques of monotone systems to study a general model of gene expression. Then we consider a model of a loop where the polymerase allows the transcription of the gene of polymerase. This model is not monotone, but based on the parameter values provided by biologists, we have reduced it to a simple and monotone model. The study of the reduced system shows that the full system can have either a single equilibrium point at the origin which is globally stable or there is another one stable strictly positive equilibrium and the origin is locally unstable. The alternative between these two cases depends on the total amount of the concentration of ribosomes. We then study a general model of the genetic machinery, taking the model studied previously for the polymerase and coupling it with a model for the synthesis of ribosomes. We finally apply different types of qualitative controls on models of small nonlinear gene networks to stabilize for example an unstable equilibrium point or to generate a limit cycle instead of a stable equilibrium.

Résumé

Le but de cette thèse est la modélisation, la réduction, l'analyse et la commande de systèmes biologiques. La modélisation des réseaux biologiques s'effectue par équations différentielles, les systèmes sont généralement non linéaires, de grandes dimensions, avec différentes échelles de temps et complexes à analyser. Tout d'abord, en utilisant des techniques de systèmes monotones et compartimentaux, nous étudions la stabilité globale de l'équilibre du modèle enzymatique de Michaelis-Menten sans aucune approximation, dans les deux cas où le système est fermé et ouvert ; nous étudions aussi le cas général d'une chaîne de réactions enzymatiques. Les réseaux biologiques sont composés généralement d'une partie génétique et d'une partie métabolique en interaction, nous étudions donc différents types de modèles de couplage des chaînes de réactions métaboliques avec la partie génétique ; nous réduisons le système en se basant sur la différence des échelles de temps (théorème de Tikhonov). Dans une deuxième partie, nous appliquons les mêmes techniques de systèmes monotones pour étudier un modèle général d'expression d'un gène. Puis nous considérons un modèle en boucle où la polymérase permet la transcription du gène de la polymérase. Ce modèle n'est pas monotone, mais en se basant sur des valeurs de paramètre fournis par des biologistes, nous l'avons réduit à un modèle plus simple et monotone. L'étude du système réduit montre que le système complet peut avoir soit un seul point d'équilibre à l'origine qui est globalement stable ou bien il existe un autre point d'équilibre strictement positif stable et l'origine est localement instable. L'alternative entre ces deux cas dépend de la quantité totale de la concentration des ribosomes. Nous étudions ensuite un modèle général de la machinerie génétique, en prenant le modèle étudié précédemment pour la polymérase et en le couplant avec un modèle pour la synthèse des ribosomes. Nous avons appliqué à la fin différents types de contrôles qualitatifs sur des modèles de petits réseaux génétiques non linéaires pour stabiliser par exemple un point d'équilibre instable ou pour avoir un cycle limite au lieu d'un équilibre stable.

Articles

1. I. Belgacem and J.-L. Gouzé, "Global stability of full open reversible Michaelis-Menten reactions," IFAC Symposium on Advanced Control of Chemical Processes, Singapore, July 10-13, 2012.

2. I. Belgacem and J.-L. Gouzé, "Global stability of enzymatic chain of full reversible michaelis-menten reactions," Acta Biotheoretica, 2013, $10.1007/s10441 - 013 - 9195 - 3$.

3. I. Belgacem and J.-L. Gouzé, "Stability analysis and reduction of gene transcription models," in 52nd IEEE Conference on Decision and Control (CDC'13), Florence, Italy, 2013.

4. I. Belgacem and J.-L. Gouzé, "Analysis and reduction of transcription translation coupled models for gene expression," in CAB (Computer applied to Biotechnology). Mumbai, India: IFAC, 2013.

5. I. Belgacem and J.-L. Gouzé, "Mathematical study of the global dynamics of a concave gene expression model," in MED'14, 22nd Mediterranean Conference on Control and Automation. Palermo, Italy, 2014.

6. I. Belgacem, E. Grac, D. Ropers, and J.-L. Gouzé. Stability analysis of a reduced transcription-translation model of RNA polymerase. In Proceedings of CDC 14, Los Angeles, California, USA. December 2014.

7. I. Belgacem and Jean-Luc Gouzé. Qualitative control of genetic oscillators, submitted to Acta Biotheoretica, 2015.

8. I. Belgacem, E. Grac, D. Ropers, and J.-L. Gouzé. Time scales reduction and stability analysis of the transcription-translation model of RNA polymerase, to be submitted to Automatica, 2015.

Contents

Chapter 1

Modélisations des réactions métaboliques et réseaux de régulations génétiques

1.1 Introduction

Nous présentons dans ce chapitre les principes de base dont nous aurons besoin sur la modélisation des réseaux de régulation génétique. Pour comprendre les modèles mathématiques que nous aborderons, on présente quelques aspects de la biologie, en commençant par définir ce que veut dire une cellule, les éléments qu'elle contient, les réactions enzymatiques et surtout expliquer le dogme central de la biologie moléculaire. Puis nous traitons la modélisation des réactions enzymatiques en présentant un exemple (Michaelis-Menten) et nous montrons après comment les réseaux de régulation génétique sont modélisés mathématiquement en utilisant des équations différentielles continues ou continues par morceau au cours du temps.

1.2 Principe de régulation cellulaire

Une cellule est l'unité de base de tout organisme vivant, voir [61], [45]. L'invention de la microscopie a permis l'observation des compartiments à l'intérieur de la cellule. La théorie cellulaire est élaborée par Schleiden et Schwann en 1839, voir [67]. La figure 1.2.1 présente une cellule et les éléments qu'elle contient. Sa membrane lui permet les échanges avec le milieu extérieur, par exemple de se nourrir; elle prend de manière sélective de son environnement des produits (oxygène...) et rejette les déchets de son métabolisme. Sur

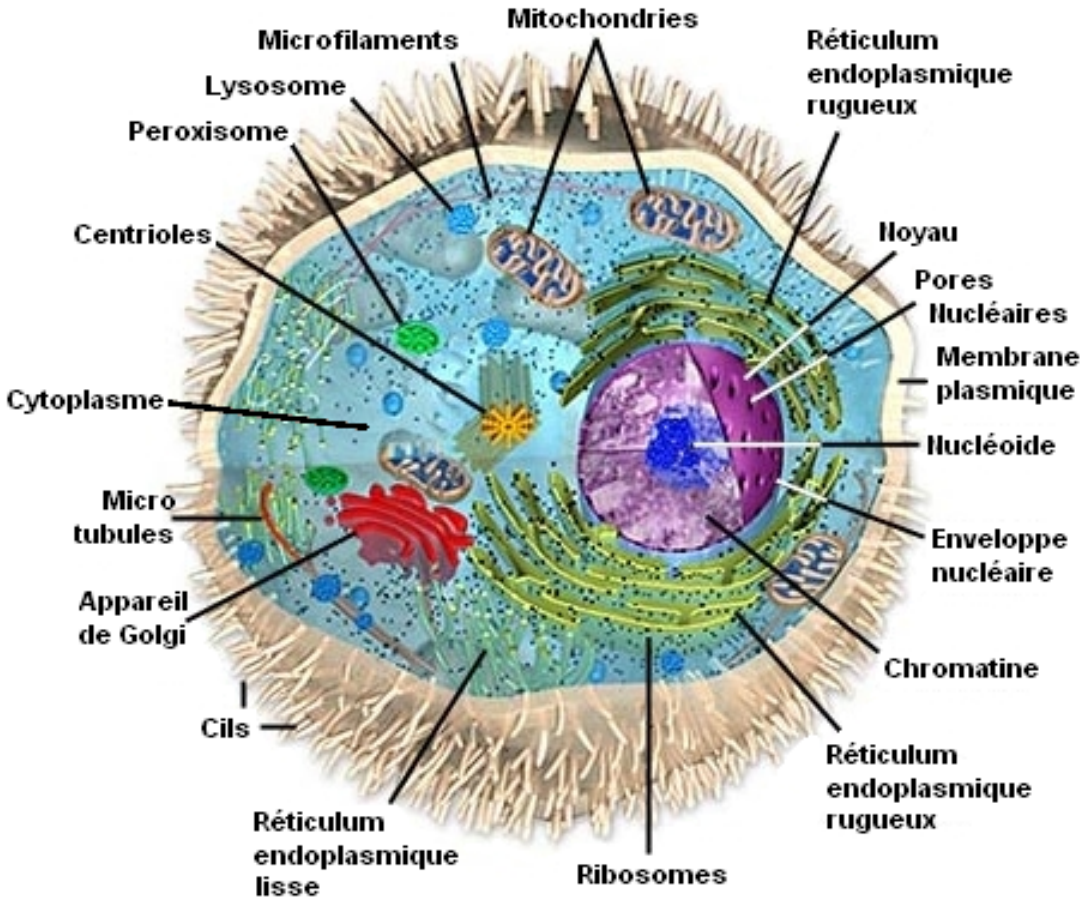

FIGURE 1.2.1: Une cellule et ces compartiments

le plan biochimique, la cellule est principalement constituée d'oxygène, d'hydrogène, de carbone et d'azote, éléments que l'on retrouve dans la majorité des composants organiques.

Le métabolisme dans la cellule représente des transformations chimiques catalysées par des enzymes. Un catalyseur (enzyme) est une substance qui accélère la vitesse d'une réaction biochimique sans être altérée dans le processus. Des centaines de réactions de ce type se produisent sans arrêt dans nos cellules qui permettent de maintenir l'organisme en vie, par exemple d'assurer la respiration, les battements de cœur, l'alimentation du cerveau, la température corporelle, la digestion...

La cellule contient aussi le génome (ADN), des organites (réticulum endoplasmique,...) et de nombreuses molécules biologiques. Suivant le type de la cellule choisi les éléments qu'elle contient peuvent varier, voir [11] pour les compositions de deux cellules différentes bactérienne (Escherichia coli) et animale (foie de rat). Pour comprendre comment fonctionne une cellule, il reste beaucoup de questions ouvertes et autant d'inconnues qui peuvent jouer un rôle peut-être important.

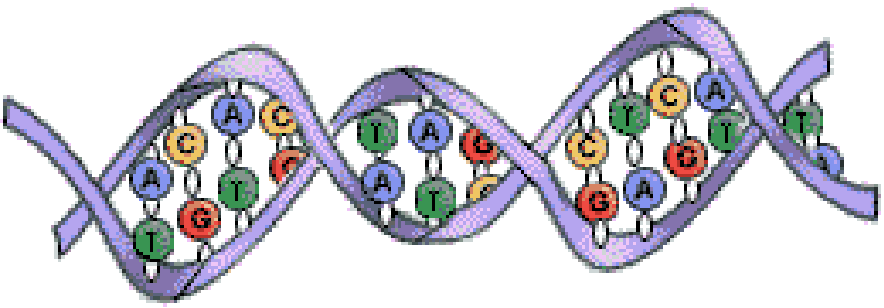

FIGURE 1.2.2: La structure de la molécule d'ADN

1.2.1 Le génome (ADN)

Les gènes sont des unités purement fonctionnelles de l'ADN (acide désoxyribonucléique), qui représentent la plus petite unité d'information génétique. La découverte de la structure de l'ADN bouleverse l'étude des phénomènes biologiques en introduisant la dimension moléculaire. Les deux points fondamentaux de la structure sont les suivants:

- les bases ou nucléotides (A, C, G, T) s'organisent en paires $A \equiv T$ et $G \equiv C$.
- Un enroulement quasi-parfait en hélice droite des deux chaînes désoxyribose-phosphate portent ces nucléotides; la structure est stabilisée par l'interaction (liaisons hydrogène) entre les bases et l'empilement successif des paires de nucléotides le long de la double-hélice, voir figure 1.2.2.

1.2.2 Le dogme central de la biologie cellulaire

La synthèse du protéine dans une cellule est l'assemblage des acides aminés présents dans son cytoplasme et guidé par l'information contenue dans l'ADN. Elle se déroule en deux temps, voir figure 1.2.3. Le passage du gène à la protéine se fait en deux étapes : tout d'abord le segment de la molécule d'ADN correspondant au gène est copié sur un brin d'ARN, que l'on appelle ARN messager (ARNm). Puis ce brin d'ARNm est à son tour recopié, mais dans un langage différent, celui des acides aminés, pour donner la séquence correspondant à la protéine synthétisée. Ces deux étapes sont appelées respectivement la transcription de l'ADN en ARN messager et la traduction de l'ARN messager en protéine. Pour la transcription on garde le même alphabet, il s'agit donc simplement de recopier le gène sur un seul brin. La seconde étape, par contre, est une vraie traduction car on passe de l'alphabet à quatre lettres de l'ADN à l'alphabet des acides aminés.

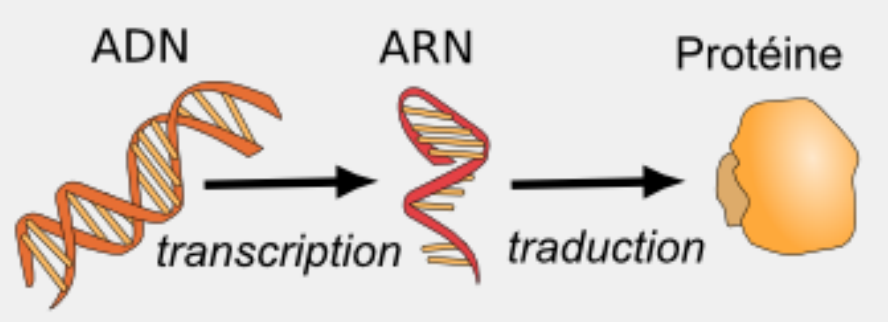

FIGURE 1.2.3: Dogme-central de la biologie cellulaire

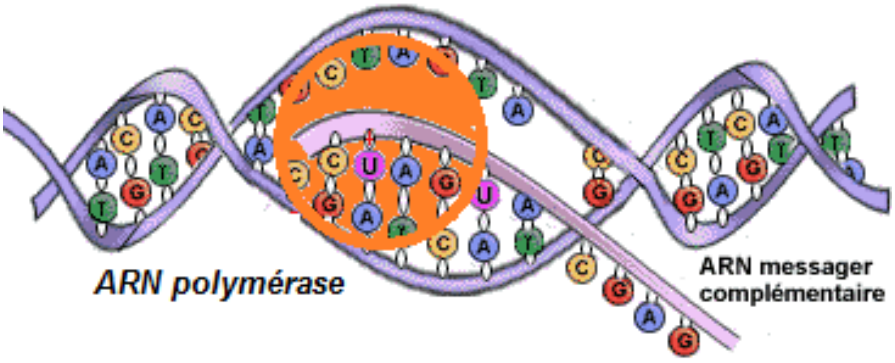

FIGURE 1.2.4: La transcription, un brin d'ADN sert à la synthèse de l'ARN messager

1.2.3 La transcription de l'ADN en ARN messager

Les gènes déterminent le code des protéines par un processus de transcription en ARNm (acide ribonucléique), voir figure 1.2.4. L'acide ribonucléique messager (ARNm) est une copie transitoire d'une portion de l'ADN correspondant à un ou plusieurs gènes. Cette recopie se fait par l'intermédiaire d'une enzyme appelée ARN-polymérase. L'ARN-polymérase doit reconnaître le commencement du gène portant l'information permettant de synthétiser la protéine voulue. Ceci se fait grâce à un site spécifique sur la molécule d'ADN appelé promoteur, qui se situe juste avant le début du gène. Un promoteur typique comporte une soixantaine de paires de base. Une fois que la polymérase s'est liée au promoteur, elle commence la transcription du gène : elle ouvre d'abord la double hélice (au niveau des liaisons hydrogène) puis elle avance le long d'un des deux brins en synthétisant le brin complémentaire. Arrivée à la fin du gène, une séquence spécifique provoque le détachement de la polymérase du brin d'ARN. La liaison de la polymérase au promoteur peut être favorisée ou inhibée par des protéines régulatrices fabriquées par d'autres gènes.

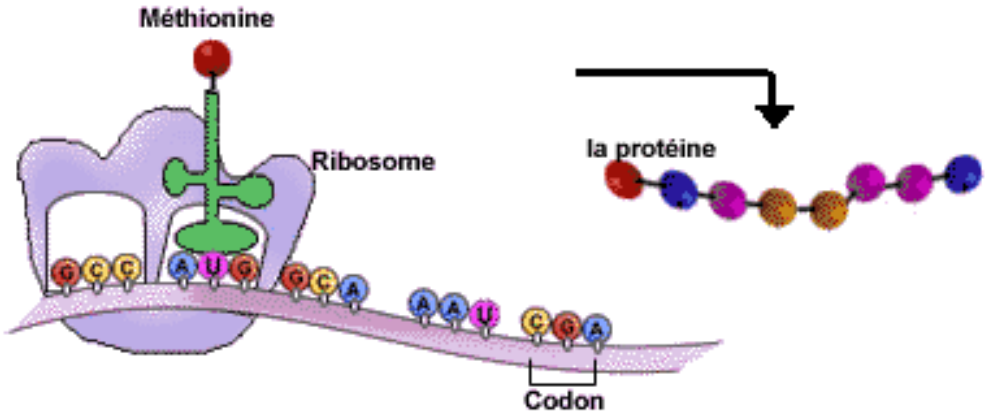

FIGURE 1.2.5: La traduction, la synthèse de la protéine à partir d'une chaîne d'acides aminés

1.2.4 La traduction de l'ARN messager en protéine

L'ARNm est utilisé comme intermédiaire par les cellules pour la synthèse des protéines. Les ARNm maturés sont ensuite traduits en protéines par les ribosomes dans le cytoplasme. Le ribosome se fixe d'abord dans la région amont de l'ARNm et glisse jusqu'au codon de démarrage. Cette étape nécessite l'intervention d'un ensemble de protéines spécifiques appelées facteurs d'initiation. Le ribosome assemblé démarre la traduction en recrutant alors le premier ARN de transfert. La figure 1.2.5 résume la traduction en montrant ses principaux acteurs.

1.3 La modélisation des réactions enzymatiques

Une réaction chimique est une transformation d'espèces chimiques (molécules, atomes ou ions) en d'autres espèces chimiques, généralement est liée à un mélange de deux espèces ou plus qui réagissent entre eux (les réactifs) pour disparaître après pour former au cours de la réaction les produits de la réaction. Au cours d'une réaction chimique il y a conservation de la matière: chacun des atomes des réactifs se retrouve dans les produits (rien ne se perd), chaque atome des produits provient nécessairement d'un réactif (rien ne se crée). La masse totale des produits est égale à la masse totale des réactifs. Le temps consacré pour la transformation des réactifs en produit dépend de la vitesse le la réaction.

Considérons par exemple la réaction chimique suivante:

$$S + \ C \xrightarrow{k} \ P. \tag{1.3.1}$$

Suivant la loi d'action de masses établie par Guldberg et Waage : à une température constante, la vitesse des réactions est proportionnelle au produit des concentrations des réactifs. Alors la vitesse de la réaction ci-dessus 1.3.1 est donnée par:

$$v = \frac{d[P]}{dt} = k[S][C]$$

où k représente la constante de vitesse, $[S]$, $[C]$ et $[P]$ représentent respectivement les concentrations des réactifs S, C et de produit P.

Supposons maintenant que les réactifs possèdent des coefficients stoechiométriques alors la réaction 1.3.1 devient:

$$\alpha \ S + \ \beta \ C \xrightarrow{k} \ P. \tag{1.3.2}$$

avec α, et β des constantes, dans ce cas la vitesse est donné par :

$$v = \frac{d[P]}{dt} = k[S]^{\alpha}[C]^{\beta}$$

où chaque réactif est élevé à une puissance égale à son coefficient stoechiométrique.

La réaction 1.3.1 se fait dans un sens. Les réactions chimiques peuvent aussi être réversibles, pouvant se faire dans le sens inverse où le produit peut se transformer aussi en réactifs comme le montre le schéma ci-dessous:

$$\alpha \ S + \ \beta \ C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \ P. \tag{1.3.3}$$

qui est équivalent à ces deux réactions élémentaires

$$\begin{aligned} \alpha \ S + \ \beta \ C &\xrightarrow{k_1} \ P. \\ P &\xrightarrow{k_{-1}} \alpha \ S + \ \beta \ C. \end{aligned} \tag{1.3.4}$$

dans ce cas la vitesse dans le sens direct s'écrit:

$$v_{direct} = \frac{d[P]}{dt} = k[S]^{\alpha}[C]^{\beta} - k_{-1}[P]$$

et la vitesse dans le sens inverse est donnée par:

$$v_{inverse} = \frac{d[S]}{dt} = \frac{d[C]}{dt} = -k[S]^{\alpha}[C]^{\beta} + k_{-1}[P].$$

Remarquons qu'il y a conservation de la matière : la somme totale reste fixe: $v_{direct} + v_{inverse} = 0$.

Une réaction enzymatique est une réaction de type chimique catalysée par une enzyme (biochimique).

Une enzyme est une protéine fabriquée par l'organisme qui permet l'accélération de réactions chimiques. Elles jouent un rôle dans toutes les fonctions, comme la digestion avec des enzymes intervenant pour décomposer les grosses molécules en substrats plus petits qui pourront être incorporés par l'organisme.

Pour une réaction enzymatique simple n'impliquant qu'une seule enzyme, voir la figure 1.3.1, la présence d'une enzyme E provoque l'augmentation de la vitesse de la réaction, et E est inchangée à la fin de la réaction. La molécule transformée au cours d'une réaction enzymatique s'appelle le substrat noté S et la molécule résultante de la transformation du substrat s'appelle le produit noté P.

Une réaction enzymatique se déroule en deux étapes:

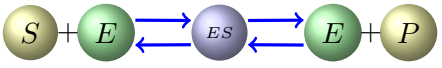

FIGURE 1.3.1: Une réaction enzymatique simple impliquant une seule enzyme.

- formation du complexe enzyme-substrat (ES)
- catalyse : succession de réactions intermédiaires permettant la transformation du substrat en produit.

1.4 L'équation de vitesse du mécanisme irréversible de Michaëlis-Menten

Le mécanisme d'une réaction enzymatique, a été proposé par Henri (1903), et après par Michaelis et Menten (1913), voir [50] . Considérons la réaction irréversible constituée d'un substrat noté S, d'une enzyme notée E et d'un produit noté P présentée ci-dessous:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} P + E$$

Suivant la loi d'action de masse, la représentation mathématique de l'évolution de ce modèle est donnée par ce système:

$$\begin{cases} \dfrac{ds}{dt} = -k_1 se + k_{-1} c \\ \dfrac{de}{dt} = -k_1 se + k_{-1} c + k_2 c - k_{-2} pe \\ \dfrac{dc}{dt} = k_1 se - k_{-1} c - k_2 c \\ \dfrac{dp}{dt} = k_2 c \end{cases} \tag{1.4.1}$$

avec s, c, e et p représentent respectivement les concentrations de S, E, ES et P.

Remarquons que la quantité d'enzyme sous sa forme libre et complexe 'enzyme-substrat' reste constant au cours de temps c'est à dire:

$$\dot{e} + \dot{c} = 0 \tag{1.4.2}$$

Notons $e + c = E_0$ avec E_0 une constante positive, on peut écrire :

$$e = E_0 - c \tag{1.4.3}$$

En remplaçant e par $E_0 - c$ dans 1.4.1, on peut avoir ce système simplifié :

$$\begin{cases} \dot{s} = -k_1 s(E_0 - c) + k_{-1} c \\ \dot{c} = k_1 s(E_0 - c) - k_{-1} c - k_2 c \\ \dot{p} = k_2 c \end{cases} \tag{1.4.4}$$

En 1913, Michaelis et Menten résolvent ce système réactionnel en posant les hypothèses simplificatrices suivantes, suggérées par les études expérimentales, voir par exemple [36]:

- La concentration totale du substrat $[S_0]$ (la concentration du substrat à l'instant initial) est très grande (>>) devant celle de l'enzyme $[E_0]$.

- Dès l'addition de l'enzyme dans la solution de substrat, il s'établit un équilibre rapide entre les formes libres de l'enzyme, du substrat et du complexe (appelé complexe de Michaëlis), on parle d'hypothèse du quasi-équilibre ou pré-équilibre, ce qui veux dire:
$$\frac{dc}{dt} = 0.$$

Mathématiquement, cela veut dire que la dynamique ou l'évolution de la variable c est très rapide en comparaison avec les autres variables. Pour voir ce qui est rapide et lent nous pouvons adimensionner le système 1.4.4 par rapport à S_0 et E_0; faisons par exemple les changements de variables: $s = xs_0$ et $c = yE_0$, on obtient ce système:

$$\begin{cases} \dot{x} = -k_1 x E_0(1-y) + \dfrac{E_0}{s_0} k_{-1} y \\ \dot{y} = k_1 x s_0(1-y) - (k_{-1}+k_2)y \\ \dot{p} = k_2 E_0 y \end{cases} \tag{1.4.5}$$

Les dynamiques de x, et y sont indépendantes de p, alors nous pouvons ignorer la dernière équation en p qui représente juste une sortie du premier sous-système en x, et y, d'où on obtient:

$$\begin{cases} \dot{x} = \dfrac{E_0}{s_0}(-k_1 x s_0(1-y) + k_{-1} y) \\ \dot{y} = k_1 x s_0(1-y) - (k_{-1}+k_2)y \end{cases} \tag{1.4.6}$$

Posons: $\mu = \frac{E_0}{s_0}$, le système 1.4.6 devient:

$$\begin{cases} \dot{x} = \mu(-k_1 x s_0(1-y) + k_{-1} y) \\ \dot{y} = k_1 x s_0(1-y) - (k_{-1}+k_2)y \end{cases} \tag{1.4.7}$$

Prenons une nouvelle échelle de temps $\tau = t\mu$, nous obtenons ce système:

$$\begin{cases} \dot{x} = -k_1 x s_0(1-y) + k_{-1} y \\ \dot{y} = \dfrac{1}{\mu}(k_1 x s_0(1-y) - (k_{-1}+k_2)y) \end{cases} \tag{1.4.8}$$

Suivant l'hypothèse Michaelis et Menten, au début de la réaction s_0 est très grand devant E_0 ce qui implique alors que μ dans ce cas est très petit et la dynamique du variable x est très lente par rapport à y (y correspond à la variable dont l'évolution est rapide). L'application de l'hypothèse de la quasi-stationnarité en biologie:

$$\dot{y} = 0 \Longrightarrow \frac{dc}{dt} = 0$$

est alors à prendre avec beaucoup d'attention ; elle est possible et valide seulement si premièrement l'hypothèse de Michaelis et Menten est vérifiée ($s_0 >> E_0$ ou μ très petit), et deuxièmement d'autres conditions pour la partie rapide du systèmes (y) doivent être aussi satisfaites. Ces conditions sur la partie rapide sont données par le théorème de Tikhonov, on revient dans le chapitre suivant avec plus de détails sur ce théorème.

Supposons que l'hypothèse Michaelis et Menten et les conditions du théorème de Tikhonov sont satisfaites, alors prenons: $\frac{dc}{dt} = 0$, on obtient:

$$\begin{aligned} c &= \frac{k_1 s E_0}{k_1 s + k_{-1} + k_2} \\ &= E_0 \frac{s}{s + K} \end{aligned} \tag{1.4.9}$$

avec $K = \frac{k_{-1}+k_2}{k_1}$ qui représente la constante de dissociation du complexe enzyme-substrat ES.

Remplaçant c dans la troisième équation dans 1.4.4, on obtient la vitesse d'apparition du produit qui est donnée par:

$$\begin{aligned} v = \dot{p} &= k_2 c \\ &= k_2 E_0 \frac{s}{s + K} \end{aligned} \tag{1.4.10}$$

qui est appelée l'équation de Michaëlis-Menten: le graphe de cette équation en fonction du substrat est représenté dans la figure (1.4.1) : $v_M = k_2 E_0$ est la vitesse maximale que peut atteindre la réaction lorsque la concentration en substrat s est très grande, K s'appelle aussi la constante de Michaelis Menten.

Pour la mesure de la vitesse initiale Michaëlis-Menten font aussi l'hypothèse qu'elle est faite pour des concentrations de produit très faible: $[P] \approx 0$ et $[S] \approx [S_0]$. Cette hypothèse permet de négliger la réaction élémentaire inverse $P + E \overset{k_{-2}}{\rightharpoonup} ES$, mais cela ne marche qu' au début de la réaction.

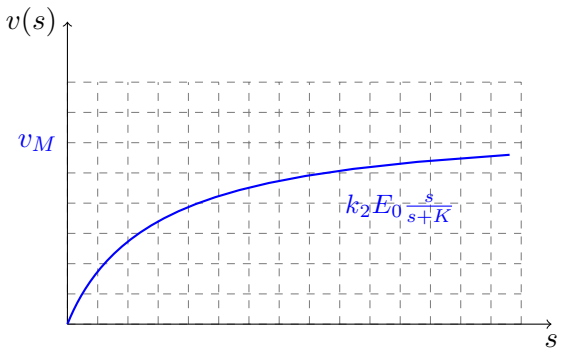

FIGURE 1.4.1: Allure de la vitesse de la réaction irréversible de Michaëlis-Menten en fonction de la concentration du substrat s

1.5 Réseaux de régulation génétique

1.5.1 Les différentes types de modélisation

Dans la littérature, on trouve différentes approches ou méthodes de modélisation qui peuvent être utilisées pour décrire ou prédire le comportement d'un réseau de régulation génétique complexe [17]. Chacune d'elles est basée sur un formalisme mathématique ou informatique particulier, qui permet de représenter un réseau de régulation génétique sous forme d'un modèle. En fonction des notions mathématiques utilisées, nous nous limitons à citer quatre principales approches existantes: le recours aux concepts de la théorie des graphes, l'utilisation d'équations différentielles, les formalisations booléennes ou logique généralisée, ou encore une description stochastique des transitions entre états moléculaires. Le choix de l'un de ces modèles dépend de l'aspect biologique : chaque approche choisie se focalise sur certaines propriétés du réseau et en néglige d'autres ; la disponibilité de données biologiques impose aussi des contraintes sur le choix, car le degré de précision de la description des réseaux et le besoin d'informations sur les facteurs de régulation et leurs interactions varie d'un formalisme à un autre. Au vu des données génétiques et moléculaires actuellement disponibles, les approches qualitatives (éventuellement sur base de modèles quantitatifs) semblent particulièrement utilisées. Dans ce qui suit on essaye de présenter l'approche de modélisation par équations différentielles qui nous intéressera beaucoup plus dans cette thèse.

1.5.2 Approche de modélisation par équations différentielles

En générale c'est l'approche la plus utilisée pour modéliser les réseaux de régulation génétique (RRG), qui représente mieux la réalité. Les concentrations des différents espèces du système: les protéines, les ARNm, les composants moléculaires sont représentées

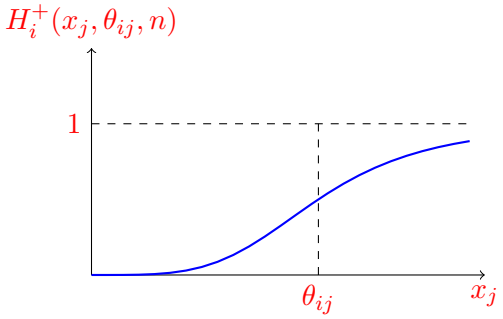

FIGURE 1.5.1: La fonction de Hill

par des variables réelles positives qui évoluent d'une manière continue ou discontinue au cours du temps.

1.5.3 Modélisation par équations différentielles continue

Le comportement d'un système de RRG est décrit par une équation différentielle dont la forme générale est la suivante:

$$\dot{x} = F(x) - Gx, \tag{1.5.1}$$

où $x \in \Re^n$ représente le vecteur d'état du système qui est le vecteur de concentrations des différentes espèces dans le milieu, $G \in \Re_+^{n\times n}$ une matrice diagonale, F est définie $\Re^n \to \Re^n$ par une fonction non linéaire, couramment elle se représente par des combinaisons de fonction de Hill, voir par exemple la figure 1.5.1: dans ce cas son expression est donnée par:

$$H_i^+(x_j, \theta_j, n) = \frac{x_j^n}{x_j^n + \theta_{ij}^n}$$

où $\theta_{ij} > 0$ représente le seuil au-delà duquel l'influence de l'espèce j sur l'espèce i change de régime, et $n > 0$ définit la raideur du saut effectué par la concentration de la protéine i après franchissement du seuil θ_{ij}.

Cette fonction prend ses valeurs dans l'intervalle [0, 1] et croit simultanément avec la croissance de la concentration x_j. Il s'agit donc d'une fonction d'induction qui augmente le taux d'expression de i si x_j augmente.

Afin d'exprimer la situation opposée, la fonction de régulation $H_i^+(x_j, \theta_{ij}, n)$ est remplacée par:

$$\begin{aligned} H_i^-(x_j, \theta_{ij}, n) &= 1 - H_i^+(x_j, \theta_{ij}, n) \\ &= \frac{\theta_{ij}^n}{x_j^n + \theta_{ij}^n} \end{aligned} \tag{1.5.2}$$

La fonction de Hill a une forme sigmoidale et elle doit toujours vérifier les propriétés suivantes:

- elle est strictement croissante (resp. décroissante) et vaut 0 en 0 et tend vers 1 à $+\infty$ (resp. vaut 1 en 0 et tend vers 0 à $+\infty$)
- Lorsque n tend vers $+\infty$,
  Si $x < \theta$, $\quad h^+(x, \theta, n) \to 0 \quad (resp. h^-(x, \theta, n) \to 1)$
  Si $x > \theta$, $\quad h^+(x, \theta, n) \to 1 \quad (resp. h^-(x, \theta, n) \to 0)$

1.5.3.1 Exemple d'un modèle classique d'expression génétique

Prenons cet exemple très simple d'un réseau de régulation génique sur la figure 1.5.2: un gène A est transcrit en ARNm noté m, l'étape de la transcription est représenté par la flèche rouge en pointillé. Après l'ARNm naissant (représenté par la courbe noire) est traduit en protéine p, le processus de la traduction est représentée par la flèche bleue en pointillé. On suppose aussi que la transcription du gène est régulée par la protéine produite, la boucle de régulation est représentée par la flèche bleue continue.

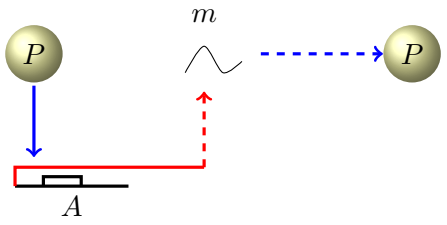

FIGURE 1.5.2: Exemple de régulation de la transcription de gène par sa protéine produit.

La représentation mathématique d'une manière classique de ce modèle ci-dessus 1.5.2 est donné par:

$$\begin{aligned} \dot{m} &= kh^+(p, \theta_p) - \gamma_m m \\ \dot{p} &= \alpha m - \beta p \end{aligned} \tag{1.5.3}$$

k, α, γ_m, β sont des constantes positives, m représente la concentration de l'ARNm, p est la concentration de la protéine. Nous supposons ici que l'ARNm et la protéine

produit peuvent se dégrader proportionnellement à leur concentration, avec un taux de dégradation respectivement γ_m et β. La production de la protéine est aussi proportionnelle à la concentration de ARNm. Le terme $kh^+(p, \theta_p)$ représente la fonction de Hill croissante (régulation positive de la transcription du gène par la protéine p).

La dynamique d'ARNm est extrêmement rapides par rapport à la dynamique de la protéines (hypothèse biologique réaliste), pour plus de détails voir [1]. Alors la transcription du gène est plus rapide que la traduction de l'ARNm en protéine. L'ARNm atteindre son équilibre rapidement, en appliquant l'hypothèse du quasi équilibre pour l'ARNm comme on l'a déjà présenté ci-dessus, on peut avoir:

$$m^* = \frac{k_a}{\gamma_m} h^+(p, \theta_p) \tag{1.5.4}$$

En remplaçant m par son équilibre, la dynamique de ce modèle 1.5.2 est représentée par une seule équation ci-dessous:

$$\dot{p} = k_p h^+(p, \theta_p) - \beta p \tag{1.5.5}$$

avec $k_p = \alpha \frac{k_a}{\gamma_m}$.

La transcription du gène dans cet exemple est régulée par une seule protéine, mais dans le cas générale un gène peut être régulé par plusieurs éléments. La fonction de Hill (non linéaire) est généralement difficile à manipuler surtout lorsque la dimension du systèmes est élevée. Pour représenter la complexité de modélisation avec des fonctions de Hill, nous allons dans ce qui suit voir un exemple plus complet d'un réseau de régulation génétique.

1.5.3.2 Exemple d'un modèle d'un réseau génétique oscillant

Prenons l'exemple d'un activateur-inhibiteur (oscillateur), voir la figure 1.5.3. Deux gènes a et b codent les protéines A et B, ces dernières contrôlent l'expression des gènes. Précisément, la présence de la protéine B avec une concentration supérieure à un certain seuil noté θ_2 inhibe l'expression du gène a, tandis que la protéine A active l'expression du gène b lorsqu'elle est présente avec une concentration supérieure à un certain seuil noté θ_1.

Dans ce cas nous étudions la dynamique de ce modèle de réseau de régulation génétique: nous caractérisons ces points d'équilibre et nous étudions la stabilité.

FIGURE 1.5.3: Exemple : Oscillateur 'activateur inhibiteur'

En utilisant l'équation du modèle réduit 1.5.5, le système du modèle dans la figure 1.5.3 est:

$$\begin{cases} \dot{x_1} = & k_1 h^-(x_2,\theta_2) - \gamma_1 x_1 \\ \dot{x_2} = & k_2 h^+(x_1,\theta_1) - \gamma_2 x_2 \end{cases} \Rightarrow \begin{cases} \dot{x_1} = & k_1 \dfrac{\theta_2^n}{x_2^n+\theta_2^n} - \gamma_1 x_1 \\ \dot{x_2} = & k_2 \dfrac{x_1^n}{x_1^n+\theta_1^n} - \gamma_2 x_2 \end{cases} \tag{1.5.6}$$

ce système 1.5.6 est non linéaire cela est du à l'utilisation du la fonction de Hill, on commence par chercher les points d'équilibres :

$$\begin{cases} x_1 = & \dfrac{k_1}{\gamma_1} \quad h^-(x_2,\theta_2) \\ x_2 = & \dfrac{k_2}{\gamma_2} \quad h^+(x_1,\theta_1) \end{cases} \Rightarrow \begin{cases} x_1 = & \dfrac{k_1}{\gamma_1} \quad \dfrac{\theta_2^n}{x_2^n+\theta_2^n} \\ x_2 = & \dfrac{k_2}{\gamma_2} \quad \dfrac{x_1^n}{x_1^n+\theta_1^n} \end{cases}$$

Il est très difficile de résoudre analytiquement ces équations ci-dessus, dans ce qui suit pour déterminer géométriquement le nombre des équilibres, nous traçons dans la figure 1.5.4, les intersections entre ces deux fonctions:

$$\begin{aligned} x_1 &= \frac{k_1}{\gamma_1} \quad \frac{\theta_2^n}{x_2^n+\theta_2^n} \\ x_2 &= \frac{k_2}{\gamma_2} \quad \frac{x_1^n}{x_1^n+\theta_1^n} \end{aligned}$$

Dans ce qui suit nous supposons $\theta_1 < \frac{k_1}{\gamma_1}$ et, $\theta_2 < \frac{k_2}{\gamma_2}$. Nous remarquons géométriquement que le système 1.5.6 admet un seul point d'équilibre.

Dans ce qui suit on va étudier la stabilité de ce point d'équilibre. La matrice Jacobienne du système 1.5.6 est donnée par:

$$\frac{\partial f}{\partial x} = \begin{pmatrix} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} \\ \\ \frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2} \end{pmatrix} = \begin{pmatrix} -\gamma_1 & \frac{-n x_2^{n-1}\theta_2^n}{(\theta_2^n+x_2^n)^2} \\ \\ \frac{n x_1^{n-1}\theta_1^n}{(\theta_1^n+x_1^n)^2} & -\gamma_2 \end{pmatrix}$$

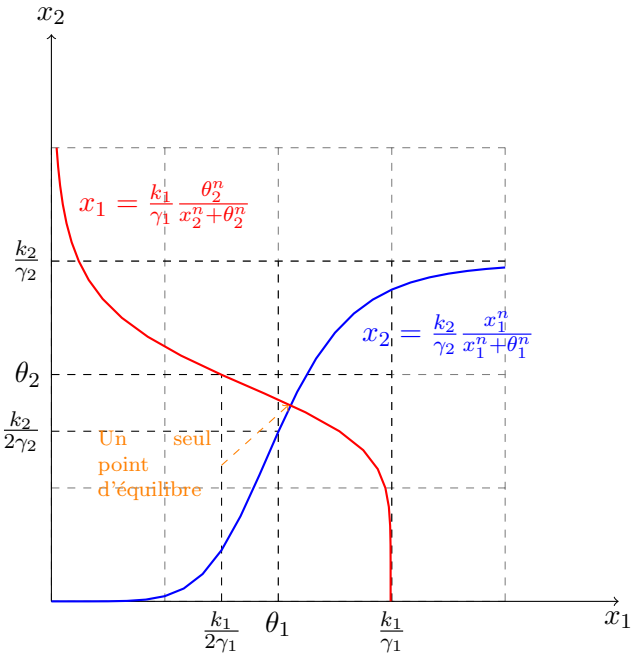

FIGURE 1.5.4: Le tracé de l'évolution de ces deux fonctions $x_1 = \frac{k_1}{\gamma_1}\frac{\theta_2^n}{x_2^n+\theta_2^n}$ et $x_2 = \frac{k_2}{\gamma_2}\frac{x_1^n}{x_1^n+\theta_1^n}$, il y a un seul point d'intersection.

Supposons que $x^* = (x_1^*, x_2^*)$ est le point d'équilibre pour le système 1.5.6, donc au voisinage de l'équilibre la matrice Jacobienne est:

$$\frac{\partial f}{\partial x}|_{x^*} = \begin{pmatrix} -\gamma_1 & \frac{-nx_2^{*n-1}\theta_2^n}{(\theta_2^n+x_2^{*n})^2} \\ \frac{nx_1^{*n-1}\theta_1^n}{(\theta_1^n+x_1^{*n})^2} & -\gamma_2 \end{pmatrix}$$

Alors quelque soit la valeur de point d'équilibre $x^* = (x_1^*, x_2^*)$, la trace est toujours négative et le déterminant est toujours positif : le système est stable au voisinage de son point d'équilibre (stabilité locale). Considérons les valeurs numériques suivantes $k_1 = 3, \gamma_1 = 0.25, k_2 = 4, \gamma_2 = 0.5, \theta_1 = 4, \theta_2 = 3, x_{01} = x_{02} = 2$, le résultat de la simulation est donné dans la figure 1.5.5:

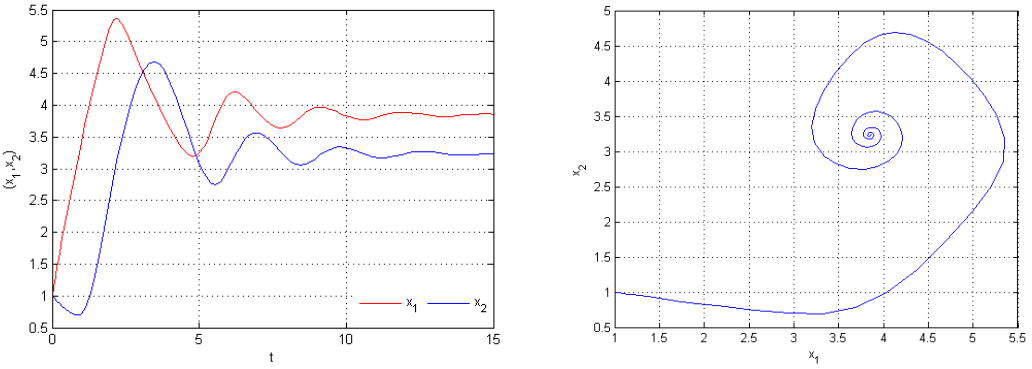

FIGURE 1.5.5: Le comportement du système oscillateur avec la modélisation par la fonction de Hill

1.6 Modélisation par équations différentielles affines par morceaux

Lorsque les équations différentielles non linéaires ne peuvent pas être analysées mathématiquement, alors afin de simplifier l'analyse mathématique, une approximation de la fonction de Hill continue par une fonction discontinue ou en escalier est proposée, voir [30]: elle est obtenu à partir de la fonction de Hill en faisant n tend vers $+\infty$:

$$\begin{aligned} S_i^+(x_j,\theta_{i,j}) &= \lim_{n\to+\infty} h_i^+(x_j,\theta_{i,j},n) \\ &= \begin{cases} 1 & si \quad x_j > \theta_{ij} \\ 0 & si \quad x_j < \theta_{ij} \end{cases} \end{aligned} \tag{1.6.1}$$

et,

$$\begin{aligned} S_i^-(x_j,\theta_{i,j}) &= \lim_{n\to+\infty} h_i^-(x_j,\theta_{i,j},n) \\ &= 1 - S_i^+(x_j,\theta_{i,j}) \\ &= \begin{cases} 1 & si \quad x_j > \theta_{ij} \\ 0 & si \quad x_j < \theta_{ij} \end{cases} \end{aligned} \tag{1.6.2}$$

Voir la figure 1.6.1 pour le tracé de la fonction $S_i^+(x_j,\theta_{i,j})$. Dans ce cas une classe de modèles d'équations différentielles affines par morceaux (piecewise affine systems - PWA) est employée. La variation de la concentration de chaque espèce moléculaire dépend d'un terme de production et d'un terme de dégradation.

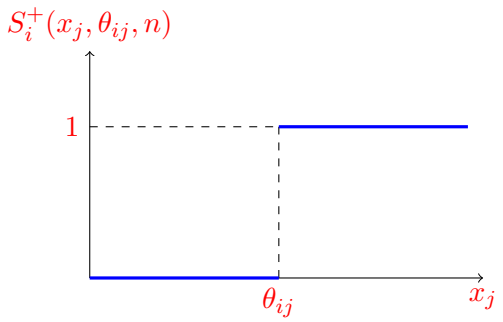

FIGURE 1.6.1: Allure de la fonction step: $S_i^+(x_j, \theta_{i,j})$.

Example 1.1. *Reprenons l'exemple précédent alors ce réseau de régulation génétique est modélisé par un système d'équations différentielles affines par morceaux (piecewise affine systems - PWA), comme suit:*

$$\begin{cases} \dot{x_1} = & k_1 S^-(x_2, \theta_2) - \gamma_1 x_1 \\ \dot{x_2} = & k_2 S^+(x_1, \theta_1) - \gamma_2 x_2 \end{cases}$$

Notons par:

- R_1 *représente la région où* $x_1 < \theta_1$ *et* $x_2 < \theta_2$,
- R_2 *représente la région où* $x_1 > \theta_1$ *et* $x_2 < \theta_2$,
- R_3 *représente la région où* $x_1 > \theta_1$ *et* $x_2 > \theta_2$,
- R_4 *représente la région où* $x_1 < \theta_1$ *et* $x_2 > \theta_2$.

Le champ de vecteurs dans ce cas est variable en fonction de la région, voir figure 1.6.2, la dynamique du système est donnée comme suit:

$$\begin{array}{llll} Si & x \in R_1 & alors & \begin{cases} \dot{x_1} = & k_1 - \gamma_1 x_1 \\ \dot{x_2} = & -\gamma_2 x_2 \end{cases} \\ Si & x \in R_2 & alors & \begin{cases} \dot{x_1} = & k_1 - \gamma_1 x_1 \\ \dot{x_2} = & k_2 - \gamma_2 x_2 \end{cases} \\ Si & x \in R_3 & alors & \begin{cases} \dot{x_1} = & -\gamma_1 x_1 \\ \dot{x_2} = & k_2 - \gamma_2 x_2 \end{cases} \\ Si & x \in R_4 & alors & \begin{cases} \dot{x_1} = & -\gamma_1 x_1 \\ \dot{x_2} = & -\gamma_2 x_2 \end{cases} \end{array} \tag{1.6.3}$$

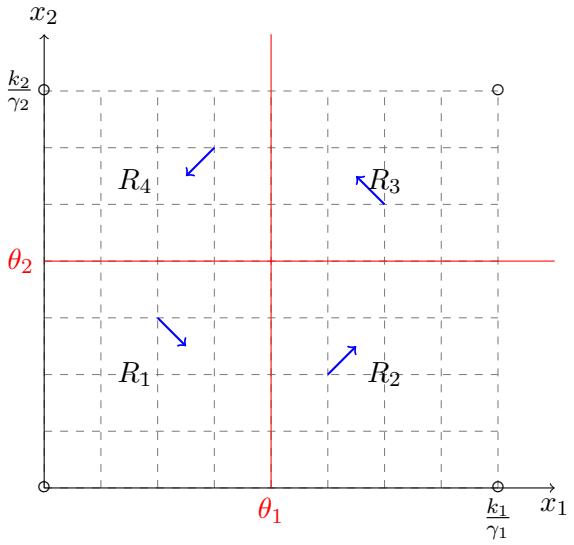

FIGURE 1.6.2: Représentation du champs de vecteur (les flèches en bleus) par rapport à chaque région dans le cas de la modélisation du système oscillateur par la fonction Step.

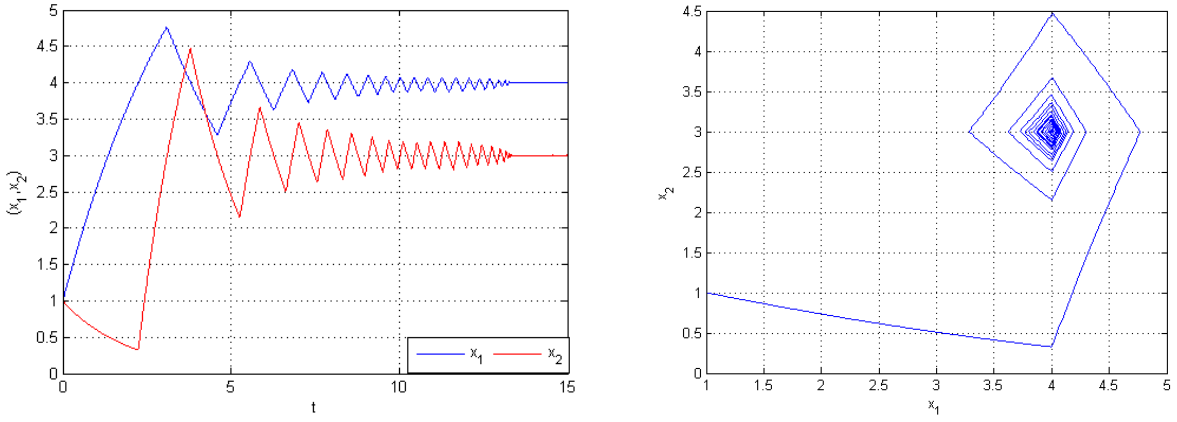

FIGURE 1.6.3: Le comportement du système oscillateur avec la modélisation par la fonction Step.

Considérons les valeurs numériques suivantes $k_1 = 2, \gamma_1 = 0.25, k_2 = 4, \gamma_2 = 0.5, \theta_1 = 4, \theta_2 = 3, x_{01} = x_{02} = 1$, *les résultats de la simulation sont donnés dans la figure 1.6.3:*

1.6.1 Les points focaux

Pour l'exemple précédent les points focaux sont représentés par des petit cercles, voir figure 1.6.2, dans chaque région $R_i, i \in \{1, 2, ..., 4\}$, le point défini par $f(R^i) = (x_1^*, x_2^*)_i$ avec:

$$\begin{cases} x_1^* = \dfrac{k_1 S^-(x_2, \theta_2)}{\gamma_1} \\ x_2^* = \dfrac{k_2 S^+(x_1, \theta_1)}{\gamma_2} \end{cases}$$

est appelé point focal pour la région R^i. Les valeurs de $S^-(x_2, \theta_2), S^+(x_1, \theta_1)$ sont variables suivant la région.

Dans le cas général deux cas peuvent se présenter, voir [33]:

- Si $f(R_i) \in R^i$, ce qui veut dire que le point focal est aussi à l'intérieur de R_i, alors dans ce cas $f(R_i)$ est un point d'équilibre asymptotiquement stable dans la région R_i : toutes les trajectoires à partir de la condition initiale $x(0)$ dans R_i ne vont pas quitter R_i et convergent vers $f(R_i)$.
- Si $f(R_i) \notin R^i$ ce qui veut dire que le point focal est à l'extérieur de R_i, alors toutes les trajectoires à partir de la condition initiale $x(0)$ dans R_i se dirigent et atteignent les frontières de R_i et tendent vers le point focal.

1.6.2 Solution au sens de Filippov au niveau des surfaces discontinues

Toute la difficulté se trouve maintenant au niveau des seuils où le système n'est pas défini. Dans la suite on va présenter une approche de Filippov pour déterminer l'évolution de système sur une surface discontinue ou ce qu'on appelle une régularisation par mode glissant. Considérons le système suivant:

$$\begin{cases} \dot{x} = f_1(x) & \text{si } h(x) > 0 \\ \dot{x} = f_2(x) & \text{si } h(x) < 0 \end{cases} \tag{1.6.4}$$

Les spécifications ci-dessus sont évidemment incomplètes puisque aucune déclaration n'est faite pour le cas où $h(x) = 0$.

La surface de discontinuité est noté $S = \{x \in \Re^n : h(x) = 0\}$. Prenons S^+ et S^- représentant respectivement l'ensemble de tous les points où $h(x)$ est positive et négative. Cependant, il n'est pas clair comment définir le champ de vecteur sur la surface de discontinuité. Dans la littérature, il y a différents concepts de solution, Filippov a proposé le théorème ci-dessous.

Theorem 1.1. *[24]*

Supposons que les régions S^+ *et* S^- *dans l'espace* $\Re^n$ *soient séparées par une surface discontinue* S*. Notons* F_{s^-}, F_{s^+} *et* F_s *les champs de vecteur définis respectivement pour* $x \in S^-, x \in S^+ et\, x \in S$*. Prenons* x *un point sur la surface de discontinuité* S *et notons* $F^-(x)$ *et* $F^+(x)$ *les limites de* $F(x)$ *quand le point* x *est approché des côtés opposés du plan tangent à* S*.*

Si $\frac{\partial S}{\partial x}F_{s^-} \geq 0, \frac{\partial S}{\partial x}F_{s^+} \leq 0,\ \frac{\partial S}{\partial x}F_{s^-} - \frac{\partial S}{\partial x}F_{s^+} > 0$*. Alors pour que* $x(t) \in S$ *puisse être une solution il faut que :*

$$\frac{dx}{dt} = \dot{x}(t) = \alpha\ F^+(x) + (1-\alpha)\ F^-(x),$$

ou $\alpha \in [0,1]$ *tel que le champ de vecteur*

$$F_s = \alpha\ F^+(x) + (1-\alpha)\ F^-(x),$$

soit tangent à la surface de discontinuité S*, c'est-à-dire,*

$$\dot{S}(x) = \frac{\partial S}{\partial x}F_s = 0 \Rightarrow \alpha = \frac{\frac{\partial S}{\partial x}F_{s^-}}{\frac{\partial S}{\partial x}F_{s^-} - \frac{\partial S}{\partial x}F_{s^+}}$$

Par l'application du théorème ci dessus on réécrit les équations 1.6.4 comme:

$$\dot{x} \in F(x)$$

où la fonction $F(x)$ est définie par :

$$F(x) = \begin{cases} f_1(x) & si\ h(x) > 0 \\ f_2(x) & si\ h(x) < 0 \\ \alpha\ f_1(x)\ + (1-\alpha)\ f_2(x) & si\ \ h(x) = 0 \end{cases}$$

avec $\alpha \in [0,1]$ donné par :

$$\alpha = \frac{\frac{\partial h}{\partial x}f_2(x)}{\frac{\partial h}{\partial x}f_2(x) - \frac{\partial h}{\partial x}f_1(x)}$$

Remarquons que le système dynamique discontinu donné par les équations 1.6.4 a été reformulé comme inclusion différentielle à l'aide de la solution proposée par Filippov. Le champ de vecteur sur la surface de discontinuité appartient à l'enveloppe convexe des champs de vecteurs $f_1(x)$ et $f_2(x)$. Dans ce cas l'équation $\dot{x}(t) = \alpha F^+(x) + (1-\alpha)F^-(x)$

définit l'évolution du système sur la surface de discontinuité $S = \{x \in \Re^n : h(x) = 0\}$, et s'appelle mode glissant par la solution de Filippov.

Prenant par exemple les deux régions R_1 et R_2 qui sont séparées par un mur noté par exemple W (surface de discontinuité), le champ de vecteur est continu à l'intérieur de chaque régions R_1 et R_2. Par contre l'évolution de ce système sur le mur W n'est pas défini. La surface de discontinuité dans ce cas est définie par $S_1 = \{x \in \Re^n : x_1 - \theta_1 = 0\}$. Donc suivant le théorème ci-dessus proposé par Filippov si $\frac{\partial S_1}{\partial x} f_{R_1} \geq 0, \frac{\partial S_1}{\partial x} f_{R_2} \leq 0$, $\frac{\partial S_1}{\partial x} f_{R_1} - \frac{\partial S_1}{\partial x} f_{R_2} > 0$, le champ de vecteur sur la surface de discontinuité sera égal à l'enveloppe convexe suivante: $f_{S_1} = \alpha f_{R_2} + (1 - \alpha) f_{R_1}$, avec f_{R_1} et f_{R_2} représente respectivement le champ de vecteur dans la région R_1 et R_2.

Nous avons:

$$f_{R_1} = \begin{pmatrix} k_1 - \gamma_1 x_1 \\ -\gamma_2 x_2 \end{pmatrix} \tag{1.6.5}$$

et,

$$f_{R_2} = \begin{pmatrix} k_1 - \gamma_1 x_1 \\ k_2 - \gamma_2 x_2 \end{pmatrix} \tag{1.6.6}$$

Remarquons que dans ce cas $\frac{\partial S_1}{\partial x} f_{R_1} = \frac{\partial S_1}{\partial x} f_{R_2} \geq 0$, ce qui implique que x_1 a le même sens de variation dans les deux régions séparées par le mur W. Donc les trajectoires traversent à travers le mur sans problème en passant de la région R_1 à l'autre région R_2, ce dernier est appelé un mur transparent.

Si $\frac{\partial S_1}{\partial x} f_{R_1} \leq 0$ et $\frac{\partial S_1}{\partial x} f_{R_2} \geq 0$, cela signifie que les trajectoire s'éloignent de la surface discontinue sur les deux frontières opposés. Dans ce cas on parle d'un mur blanc où toutes les trajectoire quitteront la surface discontinue. Pour le cas contraire c'est à dire le cas où les conditions de Filippov sont vérifiées on parle d'un mur noir où toutes les trajectoires doivent rester sur la surface discontinue. Pour chaque cas mur noir, blanc ou transparent, nous représentons dans la figure 1.6.4 les directions des champs de vecteurs.

1.6.3 Point d'équilibre au sens de Filippov

Notons ϕ le point d'intersection des droites $x_1 = \theta_1$ et $x_2 = \theta_2$. Suivant Filippov le champs de vecteurs en ce point $\phi = (\theta_1, \theta_2)$ est défini par: $F(\phi) = \alpha f_{R_1} + \beta f_{R_2} + \gamma f_{R_3} + \delta f_{R_4}$, avec f_{R_3} et f_{R_4} représentant respectivement le champ de vecteur dans la région R_3 et R_4, $\{\alpha, \beta, \gamma, \delta, \} \in [0, 1]$, et $\alpha + \beta + \gamma + \delta = 1$. Le point $\phi = (\theta_1, \theta_2)$ est un point

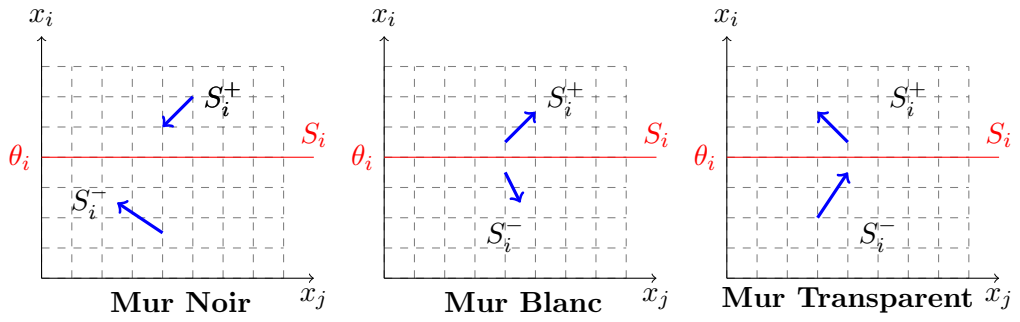

FIGURE 1.6.4: Les champs de vecteurs dans le cas d'existence d'un mur noir, blanc ou transparent, $S_i = x_i - \theta_i = 0$ représente la surface de discontinuité, S_i^+ et S_i^- représente respectivement tous les points $x_i > \theta_i$ et $x_i < \theta_i$.

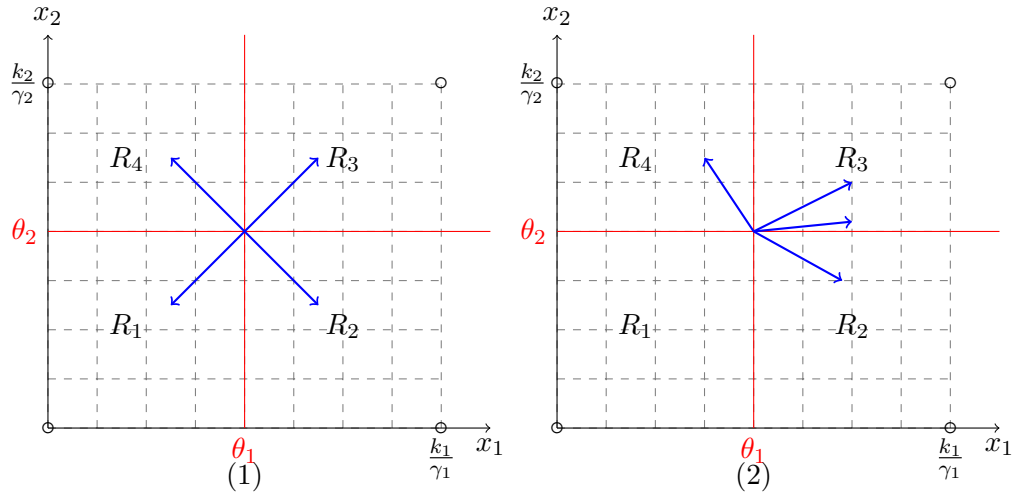

FIGURE 1.6.5: (1) représente un point d'équilibre au sens de Filippov;
(2) n'est pas un point un équilibre au sens de Filippov.

d'équilibre au sens de Filippov si $0 \in F(\phi)$, ce qui veut dire qu'il existe $\alpha, \beta, \gamma, \delta$ tel que: $F(\phi) = \alpha f_{R_1} + \beta f_{R_2} + \gamma f_{R_3} + \delta f_{R_4} = 0$. Si $0 \notin F(\phi)$ alors ϕ n'est pas un point d'équilibre au sens de Filippov. La figure 1.6.5 montre l'enveloppe convexe fermée au point ϕ par dans le cas où d'un équilibre au sens de Filippov et dans le cas où il ne représente pas un point d'équilibre au sens de Filippov.

1.6.4 Stabilité de l'équilibre au sens de Filippov

Nous donnons dans ce paragraphe quelques notions simples sur un exemple; elles nous seront utiles dans le dernier chapitre.

Dans ce qui suit nous utilisons la fonction de premier retour pour étudier la stabilité globale de l'équilibre au sens de Filippov. Pour simplifier les calculs nous faisons au

début ce changement de variable $t' = \gamma t$, $y_1 = x_1 - \theta_1, y_2 = x_2 - \theta_2$, les dynamiques du système 1.6.3 deviennent:

$$
\begin{aligned}
&Si \quad y; (y_1 < 0 \ \text{ et } \ y_2 < 0) \qquad \text{alors} \quad \begin{cases} \dot{y_1} = & \alpha - y_1 \\ \dot{y_2} = & -\theta_2 - y_2 \end{cases} \\
&Si \quad y; (y_1 > 0 \ \text{ et } \ y_2 < 0) \qquad \text{alors} \quad \begin{cases} \dot{y_1} = & \alpha - y_1 \\ \dot{y_2} = & \beta - y_2 \end{cases} \\
&Si \quad y; (y_1 > 0 \ \text{ et } \ y_2 > 0) \qquad \text{alors} \quad \begin{cases} \dot{y_1} = & -\theta_1 - y_1 \\ \dot{y_2} = & \beta - y_2 \end{cases} \\
&Si \quad y; (y_1 < 0 \ \text{ et } \ y_2 > 0) \qquad \text{alors} \quad \begin{cases} \dot{y_1} = & -\theta_1 - y_1 \\ \dot{y_2} = & -\theta_2 - y_2 \end{cases}
\end{aligned}
$$

avec $\alpha = \frac{k_1}{\gamma} - \theta_1$ et $\beta = \frac{k_2}{\gamma} - \theta_2$.

L'évolution du système à l'intérieur de chaque région au cours de temps est de la forme suivante:

$$
\begin{aligned}
\dot{y} &= A_i x + B_i \\
y(t) &= e^{A_i(t-t_0)} x(t_0) + \int_{t_0}^{t} e^{A_i(t-\tau)} B_i d\tau
\end{aligned}
$$

Prenons comme condition initiale $y_1(t_0) = y_{01} < 0$ et $y_2(t_0) = 0$, alors le système évolue jusqu'au temps t_1 suivant les équations du mouvement dans la région $y_1 < 0$ et $y_2 < 0$, avec:

$$
\begin{aligned}
y_1(t_1) &= 0 \\
y_2(t_1) &= \frac{\theta_2 \alpha}{\alpha - y_1(t_0)} - \theta_2
\end{aligned}
\tag{1.6.7}
$$

Après t_1, le système évolue jusqu'au temps t_2 dans la région $y_1 > 0$ et $y_2 < 0$ à partir de la condition initiale $y_1(t_1)$ et $y_2(t_1)$ et suivant le nouveau champ de vecteur dans cette région, avec:

$$
\begin{aligned}
y_1(t_2) &= \frac{\beta \alpha}{y_2(t_1) - \beta} + \alpha \\
y_2(t_2) &= 0
\end{aligned}
\tag{1.6.8}
$$

Après t_2, le système continue à évoluer jusqu'au t_3 suivant le nouveau champ de vecteur $y_1 > 0$ et $y_2 > 0$, à partir de la condition initiale $y_1(t_2)$ et $y_2(t_2)$, avec:

$$\begin{aligned} y_1(t_3) &= 0 \\ y_2(t_3) &= \beta - \frac{\beta\theta_1}{y_1(t_2) + \theta_1} \end{aligned} \tag{1.6.9}$$

ensuite le système continue à évoluer jusqu'au t_4 suivant les équations du mouvement dans la région $y_1 > 0$ et $y_2 < 0$, à partir de la condition initiale $y_1(t_3)$ et $y_2(t_3)$, avec:

$$\begin{aligned} y_1(t_4) &= \frac{\theta_2\theta_1}{y_2(t_3) + \theta_2} - \theta_1 \\ y_2(t_4) &= 0 \end{aligned} \tag{1.6.10}$$

Donc on peut définir maintenant le point de retour $y_1(T_1) < 0$ sur $y_2(T_1) = 0$ à partir de n'importe quelle condition initiale $y_1(T_0) < 0$ et $y_2(T_0) = 0$ comme suit:

$$\begin{aligned} y_1(T_1) = & \quad \frac{\theta_2\theta_1}{\beta + \theta_2 - \frac{\beta\theta_1}{\theta_1 + \alpha + \frac{\alpha\beta}{\frac{\theta_2\alpha}{\alpha - y_1(T_0)} - \theta_2 - \beta}}} - \theta_1 \\ = & \quad \frac{\theta_1\alpha\beta y_1(n)}{\theta_1\alpha\beta - (\theta_1\beta + \alpha\beta + \theta_1\theta_2 + \alpha\theta_2)y_1(n)} \end{aligned} \tag{1.6.11}$$

Alors pour l'évolution de y_1 on peut écrire:

$$\begin{aligned} y_1(n+1) &= f(y_1) \\ &= \frac{\theta_1\alpha\beta y_1(n)}{\theta_1\alpha\beta - \delta y_1(n)} \end{aligned}$$

avec $\delta(\theta_1\beta + \alpha\beta + \theta_1\theta_2 + \alpha\theta_2)$.

Remarquons que si $y_1(n) = 0$ alors $y_1(n+1) = 0$ et donc $y_1 = 0$ est un point fixe (au point 0, le système reste à 0) ce qui correspond aussi à $y_2 = 0$ ($x_1 = \theta_1, x_2 = \theta_2$). Pour que le système soit stable (localement) autour de ce point d'équilibre $(y_1^*, y_2^*) = (0, 0)$, il faut que:

$$|f'(0))| \leq 1. \tag{1.6.12}$$

Nous avons:

$$f'(y_1) = \left(\frac{\theta_1\alpha\beta}{\theta_1\alpha\beta - \delta y_1}\right)^2 \tag{1.6.13}$$

Donc au point $y_1^* = 0$ nous avons $|f'(0)| = 1$, ce qui veux dire que $(y_1^*, y_2^*) = (0, 0)$ est localement stable. Pour qu'il soit globalement stable, voir par exemple [30], il faut que, pour $y_1 \neq 0$:

$$|f'(y_1)| < 1$$

Ce qui est le cas ici puisque $\delta = \theta_1\beta + \alpha\beta + \theta_1\theta_2 + \alpha\theta_2$ est positif et y_1 est négatif $(0 < x_1 < \theta_1)$. Alors le système est globalement stable vers l'équilibre de Filippov $(x_1 = \theta_1, x_2 = \theta_2)$.

1.7 Conclusion

Nous avons présenté dans ce chapitre les réactions enzymatiques et les réseaux génétiques et leurs modélisations mathématiques en utilisant des équations différentielles. Pour simplifier les modèles des réseaux génétiques la fonction de Hill est remplacée par une fonction step; nous donnons des exemples pour voir l'utilité de la fonction step pour simplifier un système non linéaire sans perdre ses propriétés. La modélisation par la fonction step provoque des points de singularité où la solution n'est pas définie, pour résoudre ce problème nous présentons une solution proposé par Filippov. Nous présentons l'utilité de la fonction de premier retour pour étudier la stabilité globale d'un système continu par morceau. Dans la suite, dans le chapitre 6, nous allons remplacer les fonctions de Hill par des steps pour appliquer le contrôle qualitatif ou par région.

Chapter 2

Stability Analysis of Michaelis-Menten models and reduction of coupled metabolic-genetic systems.

We consider the closed and open Michaelis-Menten enzymatic reaction with full dynamics, i.e. not reduced with any quasi-steady state approximations. We study the corresponding dynamical system and show its global stability if the equilibrium exists. If the system is open, the equilibrium may not exist. Then we consider an open chain of reversible metabolic Michaelis-Menten reactions and also prove global stability. Our mathematical tools are monotone systems theory and compartmental systems theory.

In a second step, we consider coupled enzymatic-genetic systems. Enzymes are made by the cellular global machinery, the total concentration of enzymes is not constant and moreover the metabolites influence the production of enzymes via gene regulation. Therefore we consider different coupled metabolic-genetic models which have two time scales: "fast" and "slow" (the dynamics of the metabolic system is very fast compared to the genetic system). Using Tikhonov theorem we reduce these coupled models. Finally we study the implications of the results. We conclude that if the equilibrium of the fast subsystem (represent the dynamics of the metabolic reactions) disappears at infinity then the reduced systems using Tikhonov theorem maybe becomes not valid.

2.1 Introduction

In the field of biology, metabolic systems are an important class of dynamical systems [37]. The reactions are catalyzed by enzymes, which are proteins synthesized by genes, and metabolic and genetic systems are coupled by control loops (metabolites can regulate the synthesis of an enzyme). From a biological point of view, the study of this coupled system is of first importance [71]. Its dynamical behavior can be complex and it should be studied with mathematical models [63]. These models themselves are often large and complex, and tools for analysis or reduction are necessary, as shown in some cases studies [9, 32].

The most famous and classical enzymatic system is the (irreversible or reversible) Michaelis-Menten system. In particular, the study of the reduced system of Michaelis-Menten with the QSSA (Quasi-Steady State Approximation), based on the difference between the time scales of the reactions, led to thousand of studies [37, 53]. These studies mainly compare the behavior of the full system and that of the reduced one.

The study of the full Michaelis-Menten system stability is of great interest because this system is often used as a fast metabolic system coupled with a slow genetic system. The global stability allows to apply QSSA methods to reduce the whole system. This study also gives conditions when the QSSA methods maybe becomes not valid, if, for example, the equilibrium of the metabolic system does not exist (disappear to infinity).

Many papers have studied Michaelis-Menten approximations, but few are interested in the full system. The paper [26] studies the full closed reversible Michaelis-Menten (and gives an interesting introduction concerning the QSSA) by reducing it to a two-dimensional system. Then a phase-plane analysis is performed. Our tools are more systematic, and work even for higher dimensions (open reversible Michaelis-Menten systems cannot be reduced to two dimensions) when this two-dimensional analysis cannot be applied.

There exists other studies of metabolic chains controlled by enzymes, in other contexts [49], but, to our knowledge, none of them with our tools. For an application of monotone systems theory for chemical chains, see [19]. For a work on a similar problem of metabolic chains, with a linear approach, see [25]. Other works on metabolic chains exist, but use the reduced classical Michaelis-Menten approximation [34] or compare several approximations [15]. We believe that our tools (monotonicity, positive matrices) are well adapted to biological problems, as noticed by other studies [60]. In spite of the non linear form of kinetic rates, we are able to study the system in a simple and global way.

In the previous chapter we reduced the system of Michaelis-Menten using the QSSA hypotheses ($\frac{dc}{dt} = 0$), and we saw that this is valid only under some conditions which are not always fulfilled. In this chapter, we study the dynamical behavior of the full system of Michaelis-Menten without any approximation.

We show, with simple mathematical tools, the stability of the closed or open reversible Michaelis-Menten systems. But we also show that the open reversible Michaelis-Menten may have no equilibrium, if the input is too large.

The mathematical tools we use are known but not so classical: they belong to the theory of monotone systems, and of compartmental systems. From this point of view, we continue the work done in [48] for metabolic chains or networks with reduced enzymatic kinetics.

In a second part, we study, using the same tools and methodology, the behavior of reversible metabolic chains. We show that for a "pure" reversible enzyme system (all reactions are reversible enzymatic reactions), depending on the input, there is either no steady-state, or a single globally asymptotically stable steady-state.

In the last section, we deal with the coupled metabolic-genetic systems. We reduce its using Tikhonov theorem and briefly we give some conclusions about the validity of the reduced systems.

The mathematical notions and theorems used in this paper are briefly recalled in the appendix.

Notations: First we give some classical notations (see [51]). Consider the autonomous n-dimensional differential system

$$\dot{x} = f(x) \tag{2.1.1}$$

The notation $x(t, x_1)$, or briefly $x(t)$, corresponds to the solution starting from the initial condition x_1 parametrized by time $t \geq 0$. Function f is supposed to be continuously differentiable within some domain of interest, that will be in our case $X = \Re^n_+$. We deduce the existence and uniqueness of solutions on some time interval for the differential equation (A.1.1). Throughout the paper, we use the classical notions of Lyapunov stability.

2.2 The full closed reversible Michaelis-Menten system

In 1913, Michaelis and Menten studied the kinetics of a simple enzymatic reaction involving a single enzyme. Consider the reaction consisting of a substrate S, an enzyme E and

a product P. Michaelis and Menten proposed the following description and equations (we refer to [46] and [20]). The enzyme forms a transitory complex C before returning to its original form, giving product P from substrate S. The reactions are described by:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \overset{k_2}{\rightharpoonup} P + E$$

However, (see [16]), in principle, all reactions catalyzed by enzymes are **reversible** (similarly to chemical reactions), and this fact could play a prominent role in biochemistry. So considering the last step of the above model as a reversible reaction, the reaction scheme becomes:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E$$

The mathematical model, based on classical mass-action chemical laws, is:

$$\begin{cases} \dfrac{ds}{dt} = -k_1 se + k_{-1} c \\ \dfrac{de}{dt} = -k_1 se + k_{-1} c + k_2 c - k_{-2} pe \\ \dfrac{dc}{dt} = k_1 se - k_{-1} c - k_2 c + k_{-2} pe \\ \dfrac{dp}{dt} = k_2 c - k_{-2} pe \end{cases} \tag{2.2.1}$$

Note the two conservation laws:

$$\dot{e} + \dot{c} = 0, \quad \dot{s} + \dot{c} + \dot{p} = 0$$

This is the model of the full reversible Michaelis-Menten system, that we want to study without any approximation.

The sum of the free form and bound form of the enzyme is constant, thus we can write $e + c = E_0$, where E_0 is a positive constant, which is the total concentration of enzyme.

Therefore we can eliminate the state variable $e = E_0 - c$. Yet, we have to keep in mind that, first, E_0 depends on the initial conditions $c(0)$ and $e(0)$, and secondly that the constraint $c(t) \leq E_0$ has to be fulfilled for all time t.

The reduced system is :

$$\begin{cases} \dfrac{ds}{dt} = -k_1 s(E_0 - c) + k_{-1}c \\ \dfrac{dc}{dt} = k_1 s(E_0 - c) - k_{-1}c - k_2 c + k_{-2}p(E_0 - c) \\ \dfrac{dp}{dt} = k_2 c - k_{-2}p(E_0 - c) \end{cases} \tag{2.2.2}$$

2.2.1 Equilibrium

The first problem in this case is to show that the equilibrium always exists and fulfills the constraints on the variables that ($c^* < E_0$):

We remark that, the vector field is repulsive on the boundary of the positive orthant, see in the appendix for the definition of the invariance of the positive orthant, thus for nonnegative initial conditions, the variables stay positive for all time t, and if $c = E_0$, therefore $\dot{c} = -k_{-1}E_0 - k_2 E_0$ is negative (this point corresponds to $e = 0$) and the point is never attained, so the equilibrium $c^* \in]0, E_0[$.

To more clarify, at the equilibrium we have:

$$\begin{aligned} s^* &= \frac{k_{-1}c^*}{k_1(E_0 - c^*)} \\ p^* &= \frac{k_2 c^*}{k_{-2}(E_0 - c^*)} \end{aligned} \tag{2.2.3}$$

System (2.2.2) is closed. Thus if we take $M_0 > 0$ such that $M(s, c, p) = s + c + p = M_0$ then we obtain at equilibrium:

$$\begin{aligned} & s^* + c^* + p^* = M_0 \\ & \Rightarrow \frac{k_{-1}c^*}{k_1(E_0 - c^*)} + c^* + \frac{k_2 c^*}{k_{-2}(E_0 - c^*)} = M_0 \end{aligned} \tag{2.2.4}$$

Note that the left side of (2.2.4) is an increasing function of c^*, which is zero for c^* equals to zero, and tends to infinity when c^* tends E_0. Thus, for a fixed M_0, we deduce that (2.2.4) has a unique solution c^* such that $0 < c^* < E_0$ and which depends on plane $H_0 = \{(s, c, p) \in \Re^3_+ : s + c + p = M_0 > 0\}$. The whole steady state can be obtained by solving (2.2.4) for c^* and using (2.2.3) . It is important to notice that the equilibrium always exists (it comes from the fact that the system is closed) and is unique for each hyperplane H_0.

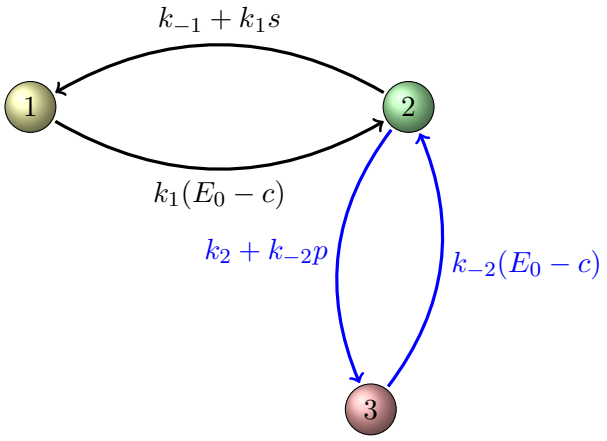

FIGURE 2.2.1: Graph of the Jacobian matrix is strongly connected

2.2.2 Global stability of the equilibrium using Lyapunov function

In the following we study the global stability of this equilibrium. The Jacobian of the system (2.2.2) matrix is $J(s, c, p)$

$$= \begin{pmatrix} -k_1(E_0 - c) & k_{-1} + k_1 s & 0 \\ k_1(E_0 - c) & -k_{-1} - k_1 s - k_2 - k_{-2}p & k_{-2}(E_0 - c) \\ 0 & k_2 + k_{-2}p & -k_{-2}(E_0 - c) \end{pmatrix} \tag{2.2.5}$$

This matrix is compartmental. We recall in the appendix the definition of a compartmental matrix, used to describe compartmental systems [39].

Remember that $c < E_0$, therefore the diagonal elements of $J(s, c, p)$ are negative and the off-diagonal elements nonnegative. Moreover, the last proposition (A.3.3) is also verified because the system is closed. Therefore the Jacobian matrix is compartmental.

The graph of the Jacobian matrix is given in figure 2.2.1, therefore it is irreducible (see appendix A.4). Then the following theorem applies:

Remark 2.1. In the case that $c = E_0$, the Jacobian matrix is not irreducible any more. But we showed above that this point is never attained.

Theorem 2.2. *Proposition 5 in [3]*
Let $M(s, c, p) = s + c + p$ the (fixed) total concentration of the closed system.
If the Jacobian matrix of the system is irreducible and compartmental, then for any $M_0 > 0$, hyperplane $H_0 = \{(s, c, p) \in \Re^3_+ : M(s, c, p) = M_0 > 0\}$ is forward invariant and contains a unique globally asymptotically stable equilibrium in H_0.

We obtain the proposition:

Proposition 2.3. *In the invariant hyperplane $M_0 = s+c+p$, the equilibrium of (2.2.2) is unique and globally asymptotically stable.*

Proof. Consider the following Lyapunov function:

$$v(s,c,p) = |\dot{s}| + |\dot{c}| + |\dot{p}| \tag{2.2.6}$$

We denote also:

$$\sigma(x) = \begin{cases} 1 & if \quad \dot{x} > 0 \\ 0 & if \quad \dot{x} = 0 \\ -1 & if \quad \dot{x} < 0 \end{cases} \tag{2.2.7}$$

Therefore, we have:

$$\begin{aligned} v(s,c,p) &= \sigma(s)\dot{s} + \sigma(c)\dot{c} + \sigma(p)\dot{p} \\ &= \begin{pmatrix} \sigma(s) & \sigma(c) & \sigma(p) \end{pmatrix} \dot{x} \end{aligned} \tag{2.2.8}$$

$\forall x$, $v(s,c,p) \geq 0$ is positive semi-definite (if $x = (s^*, c^*, p^*)$ then $v(x) = 0$);

Therefore, we have to prove that $v(s,c,p)$ is decreasing, which means that $\dot{v}(s,c,p) \leq 0$

we have:

$$
\begin{aligned}
\dot{v}(s,c,p) &= \frac{\partial v(x)}{\partial x}\dot{x} \\
&= \begin{pmatrix}\sigma(s) & \sigma(c) & \sigma(p)\end{pmatrix} J(x)\dot{x} \\
&= (\sigma(s)\frac{\partial \dot{s}}{\partial s} + \sigma(c)\frac{\partial \dot{c}}{\partial s} + \sigma(p)\frac{\partial \dot{p}}{\partial s} \quad \sigma(s)\frac{\partial \dot{s}}{\partial c} + \sigma(c)\frac{\partial \dot{c}}{\partial c} + \sigma(p)\frac{\partial \dot{p}}{\partial c} \\
&\quad \sigma(s)\frac{\partial \dot{s}}{\partial p} + \sigma(c)\frac{\partial \dot{c}}{\partial p} + \sigma(p)\frac{\partial \dot{p}}{\partial p})\dot{x} \\
&= (\sigma(s)\frac{\partial \dot{s}}{\partial s} + \sigma(c)\frac{\partial \dot{c}}{\partial s} + \sigma(p)\frac{\partial \dot{p}}{\partial s} \quad \sigma(s)\frac{\partial \dot{s}}{\partial c} + \sigma(c)\frac{\partial \dot{c}}{\partial c} + \sigma(p)\frac{\partial \dot{p}}{\partial c} \\
&\quad \sigma(s)\frac{\partial \dot{s}}{\partial p} + \sigma(c)\frac{\partial \dot{c}}{\partial p} + \sigma(p)\frac{\partial \dot{p}}{\partial p}) \begin{pmatrix}\sigma(s) & 0 & 0 \\ 0 & \sigma(c) & 0 \\ 0 & 0 & \sigma(p)\end{pmatrix}^{-1} \begin{pmatrix}\sigma(s) & 0 & 0 \\ 0 & \sigma(c) & 0 \\ 0 & 0 & \sigma(p)\end{pmatrix} \dot{x} \\
&= (\sigma^2(s)\frac{\partial \dot{s}}{\partial s} + \sigma(c)\sigma(s)\frac{\partial \dot{c}}{\partial s} + \sigma(p)\sigma(s)\frac{\partial \dot{p}}{\partial s} \quad \sigma(s)\sigma(c)\frac{\partial \dot{s}}{\partial c} + \sigma^2(c)\frac{\partial \dot{c}}{\partial c} + \sigma(p)\sigma(c)\frac{\partial \dot{p}}{\partial c} \\
&\quad \sigma(s)\sigma(p)\frac{\partial \dot{s}}{\partial p} + \sigma(c)\sigma(p)\frac{\partial \dot{c}}{\partial p} + \sigma^2(p)\frac{\partial \dot{p}}{\partial p})\,|\dot{x}|
\end{aligned}
$$

We have $\sigma^2(s) = \sigma^2(c) = \sigma^2(p) = 1$, and we have: $\frac{\partial \dot{s}}{\partial s} = -\frac{\partial \dot{c}}{\partial s} - \frac{\partial \dot{p}}{\partial s}$ ($\frac{\partial \dot{s}}{\partial s} \leq 0, \frac{\partial \dot{c}}{\partial s} \geq 0, \frac{\partial \dot{p}}{\partial s} \geq 0$), $\frac{\partial \dot{c}}{\partial c} = -\frac{\partial \dot{s}}{\partial c} - \frac{\partial \dot{p}}{\partial c}$ ($\frac{\partial \dot{c}}{\partial c} \leq 0, \frac{\partial \dot{s}}{\partial c} \geq 0, \frac{\partial \dot{p}}{\partial c} \geq 0$), and $\frac{\partial \dot{p}}{\partial p} = -\frac{\partial \dot{s}}{\partial p} - \frac{\partial \dot{c}}{\partial p}$ ($\frac{\partial \dot{p}}{\partial p} \leq 0, \frac{\partial \dot{s}}{\partial p} \geq 0, \frac{\partial \dot{c}}{\partial p} \geq 0$).

Therefore,

$$
\begin{aligned}
\dot{v}(s,c,p) = ((\sigma(c)\sigma(s) - 1)\frac{\partial \dot{c}}{\partial s} + (\sigma(p)\sigma(s) - 1)\frac{\partial \dot{p}}{\partial s} \quad (\sigma(s)\sigma(c) - 1)\frac{\partial \dot{s}}{\partial c} + (\sigma(p)\sigma(c) - 1)\frac{\partial \dot{p}}{\partial c} \\
(\sigma(s)\sigma(p) - 1)\frac{\partial \dot{s}}{\partial p} + (\sigma(c)\sigma(p) - 1)\frac{\partial \dot{c}}{\partial p})\,|\dot{x}|
\end{aligned}
\tag{2.2.9}
$$

with, $\{\sigma(s), \sigma(c), \sigma(p)\} \in \{1, -1\}$.

The system is closed ($s(t) + c(t) + p(t) = M_0$), the total concentration of the system is conserved (fixed). Therefore, it exists at least one $\sigma(x_i) \neq \sigma(x_j)$ with $i \neq j$, this means that exists at least one case that $\sigma(x_i)\sigma(x_j) = -1$, $i \neq j$. Therefore,

$$
\begin{aligned}
((\sigma(c)\sigma(s) - 1)\frac{\partial \dot{c}}{\partial s} + (\sigma(p)\sigma(s) - 1)\frac{\partial \dot{p}}{\partial s} \quad (\sigma(s)\sigma(c) - 1)\frac{\partial \dot{s}}{\partial c} + (\sigma(p)\sigma(c) - 1)\frac{\partial \dot{p}}{\partial c} \\
(\sigma(s)\sigma(p) - 1)\frac{\partial \dot{s}}{\partial p} + (\sigma(c)\sigma(p) - 1)\frac{\partial \dot{c}}{\partial p}) < 0
\end{aligned}
$$

therefore $\forall x \neq (s^*, c^*, p^*) : \dot{v}(s,c,p) < 0$, the equilibrium $x = (s^*, c^*, p^*)$ ($s^* + c^* + p^* = M_0$) is asymptotically stable.

We have also:

$$\begin{aligned} v(s,c,p) &= |\dot{s}| + |\dot{c}| + |\dot{p}| \\ &= |-k_1 s(E_0 - c) + k_{-1}c| + |k_1 s(E_0 - c) - k_{-1}c - k_2 c + k_{-2}p(E_0 - c)| \\ &+ |k_2 c - k_{-2}p(E_0 - c)|\,. \end{aligned} \tag{2.2.10}$$

So, if $\sqrt{s^2 + c^2 + p^2} \to +\infty$ then $v(s,c,p) = |\dot{s}| + |\dot{c}| + |\dot{p}| \to +\infty$. From theorem given in [47], the equilibrium $x = (s^*, c^*, p^*)$ is globally asymptotically stable.

□

The proof of this proposition for the general case (system with n dimensions, closed and the Jacobian matrix irreducible and compartmental) is given in ([39]).

See, figure (2.2.2) for the simulation of evolutions of variables (s,c,p) along the space state. Both trajectories with two different initial conditions starting from the same hyperplane are converging toward the same equilibrium.

Remark 2.4. To study the stability, we can also reduce the closed system above to the following system with only two variables:

$$\begin{cases} \dot{s} = -k_1 s(E_0 - M_0 + s + p) + k_{-1}(M_0 - s - p) \\ \dot{p} = k_2(M_0 - s - p) - k_{-2}p(E_0 - M_0 + s + p) \end{cases} \tag{2.2.11}$$

with $s + c + p = M_0$.

2.3 The full open reversible Michaelis-Menten system

Now we consider the more complex case where the system is open: there is an input u for substrate, coming from "outside", and an output for the final product, taken as a linear decay rate $-kp$. These two assumptions are classical [62]. We consider that the input u is fixed. The reaction scheme is :

$$\xrightarrow{u} S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \xrightarrow{k}$$

The differential equations describing this system are:

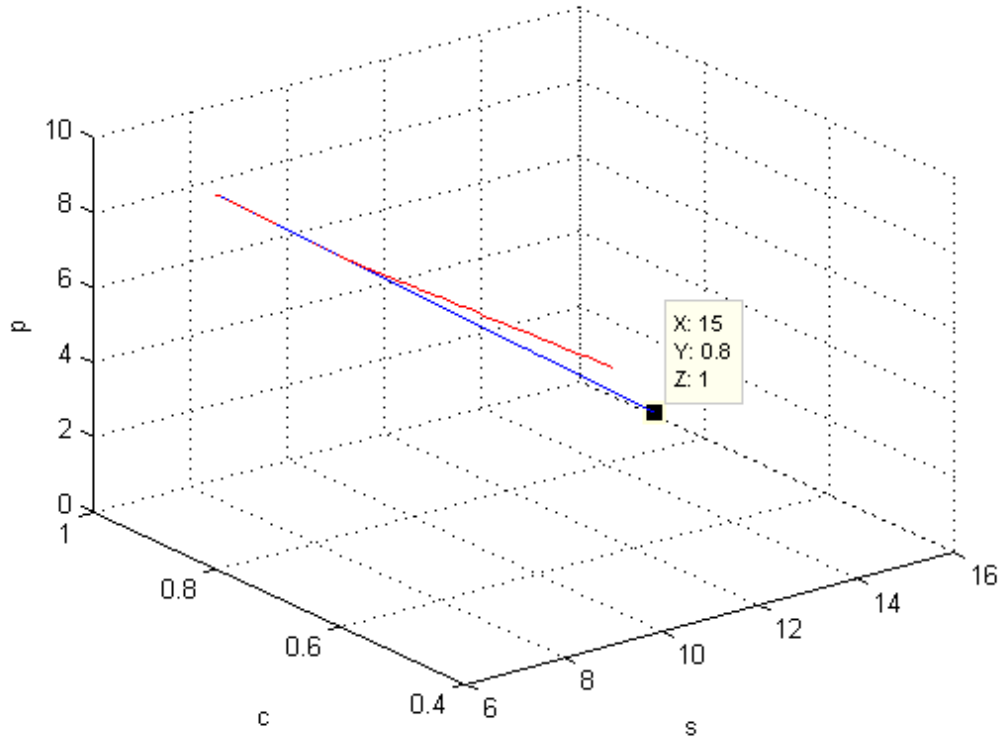

FIGURE 2.2.2: Simulations of system (2.2.2) with two different initial conditions starting from the same hyperplane $(s_{01} = 15, c_{01} = 0.8, p_{01} = 1), (s_{02} = 9, c_{02} = 0.4, p_{02} = 7.4)$ and with the parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.5, E_0 = 1$.

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 se + k_{-1}c \\ \dfrac{de}{dt} = -k_1 se + k_{-1}c + k_2 c - k_{-2}pe \\ \dfrac{dc}{dt} = k_1 se - k_{-1}c - k_2 c + k_{-2}pe \\ \dfrac{dp}{dt} = k_2 c - k_{-2}pe - kp \end{cases} \tag{2.3.1}$$

The system can be reduced in the same way as before, because the first integral $\dot{e} + \dot{c} = 0$ still holds.

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 s(E_0 - c) + k_{-1}c \\ \dfrac{dc}{dt} = k_1 s(E_0 - c) - k_{-1}c - k_2 c + k_{-2}p(E_0 - c) \\ \dfrac{dp}{dt} = k_2 c - k_{-2}p(E_0 - c) - kp \end{cases} \tag{2.3.2}$$

2.3.1 Equilibrium

Summing the three equations at equilibrium, we obtain:

$$p^* = \frac{u}{k} \tag{2.3.3}$$

The last equation gives:

$$k_2 c^* - k_{-2} p^* (E_0 - c^*) - k p^* = 0$$

therefore,

$$c^* = \frac{u + k_{-2} E_0 u/k}{k_2 + k_{-2} u/k}$$

We can compute the value s^* from the first equation of (2.3.2) to obtain:

$$s^* = \frac{u + k_{-1} c^*}{k_1 (E_0 - c^*)}.$$

But we have still to check the constraint $c^* < E_0$ and positivity of variables.

The open system (2.3.2) may admit no equilibrium, and unbounded trajectories may exist. It comes from the fact that the equilibrium disappears at infinity because $E_0 - c$ tends toward zero.

Proposition 2.5. *There exists a positive unique equilibrium* (s^*, c^*, p^*) *(with* $c^* < E_0$*) if and only if* $u < k_2 E_0$.

The biological interpretation of this proposition is that there is an equilibrium if the input is not large with respect to the total quantity of enzymes.

Proof. We have:

$$c^* = \frac{u + k_{-2} E_0 u/k}{k_2 + k_{-2} u/k}$$

This function, as a function of u, is increasing from 0 (for $u = 0$) to $E_0 + k/k_{-2}$ when u tends to infinity. This value is greater than E_0, but we have the constraint $c^* < E_0$, so the limit case is when

$$E_0 = \frac{u + k_{-2} E_0 u/k}{k_2 + k_{-2} u/k}$$

giving $u < k_2 E_0$. If this inequality is fulfilled, we can compute the value s^* to obtain:

$$s^* = \frac{u + k_{-1} c^*}{k_1 (E_0 - c^*)}.$$

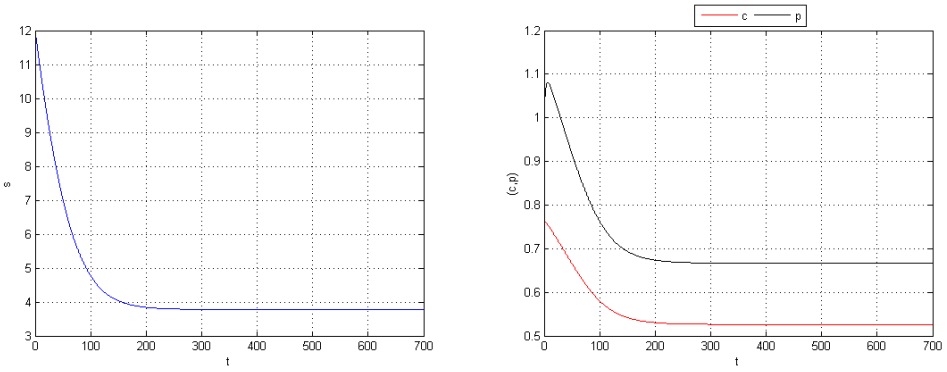

FIGURE 2.3.1: Simulation of system 2.3.2 taking these parameter values $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.5, E_0 = 1, k = 0.3, u = 0.2, s_0 = 12, c_0 = 0.8, p_0 = 1,$

This function always exists if $0 < c^* < E_0$. ■ □

The results of simulation in the case that the equilibrium exists if $u < k_2 E_0$ are given in figure (2.3.1). The evolutions of variables converge to the equilibrium which is globally stable.

Proposition 2.6. *If $u \geq k_2 E_0$, then there is no equilibrium and function $s(t) + c(t)$ is increasing and unbounded as t increases.*

Proof. The fact that there is no equilibrium is given by the preceding proposition. We write

$$\dot{s} + \dot{c} = u - k_2 c + k_{-2} p(E_0 - c).$$

Because $u \geq k_2 E_0$, and $0 \leq c(t) \leq E_0$ and $p(t) \geq 0$, then

$$\dot{s} + \dot{c} \geq k_2 E_0 - k_2 E_0 = 0$$

Moreover, if $c = E_0$, $\dot{c}$ is negative, $c(t)$ decreases, and the above inequality for $\dot{s} + \dot{c}$ is strict if $c(0) \neq E_0$. □

Biologically, it means that, if $u \geq k_2 E_0$ (the input is too large with respect to the total quantity of enzyme), then the sum $s + c$ will "explode" without bounds: the system is not viable.

For the results of simulation in the case that the equilibrium does not exist if $u \geq k_2 E_0$, see figure (2.3.2), where the subtract s explodes to infinity.

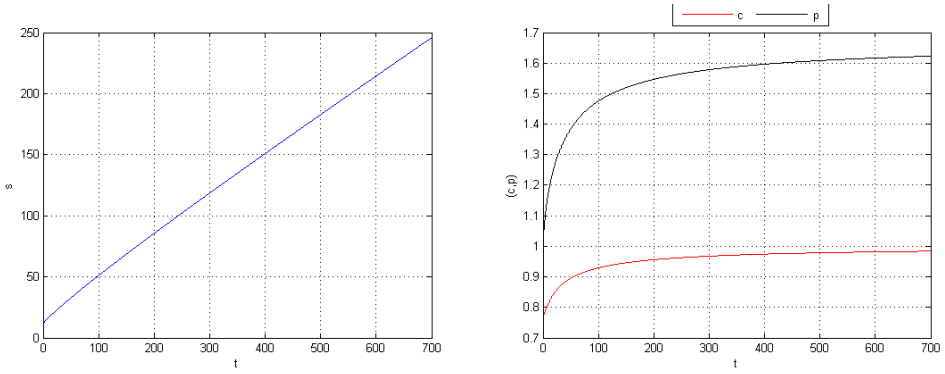

FIGURE 2.3.2: Simulation of system 2.3.2 with taking this parameter values $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.5, E_0 = 1, k = 0.3, u \geq k_2 E_0 = 0.8, s_0 = 12, c_0 = 0.8, p_0 = 1.$

2.3.2 Global stability of the equilibrium

Now we explore the case where the system does have one equilibrium (if $u < k_2 E_0$), we study in the following the global stability of this equilibrium using two different methods.

2.3.2.1 Global stability using a Lyapunov function

First, the Jacobian matrix of system (2.3.2) is:

$$J_1(s,c,p) = \begin{pmatrix} -k_1(E_0 - c) & k_{-1} + k_1 s & 0 \\ k_1(E_0 - c) & -k_{-1} - k_1 s - k_2 - k_{-2} p & k_{-2}(E_0 - c) \\ 0 & k_2 + k_{-2} p & -k_{-2}(E_0 - c) - k \end{pmatrix} \quad (2.3.4)$$

It is still compartmental, because the sum of the last column is $-k$, and therefore negative.

Proposition 2.7. *If $u < k_2 E_0$, the unique equilibrium of the open reversible Michaelis-Menten system is globally stable in $\Re^n_+$.*

Proof. We consider the same Lyapunov function as before:

$$v(s,c,p) = \begin{pmatrix} \sigma(s) & \sigma(c) & \sigma(p) \end{pmatrix} \dot{x}$$

Therefore we have:

$$\begin{aligned}\dot{v}(s,c,p) &= \frac{\partial v(x)}{\partial x}\dot{x} \\ &= \begin{pmatrix}\sigma(s) & \sigma(c) & \sigma(p)\end{pmatrix} J(x)\dot{x} \\ &= (\sigma^2(s)\frac{\partial \dot{s}}{\partial s} + \sigma(c)\sigma(s)\frac{\partial \dot{c}}{\partial s} + \sigma(p)\sigma(s)\frac{\partial \dot{p}}{\partial s} \quad \sigma(s)\sigma(c)\frac{\partial \dot{s}}{\partial c} + \sigma^2(c)\frac{\partial \dot{c}}{\partial c} + \sigma(p)\sigma(c)\frac{\partial \dot{p}}{\partial c} \\ &\quad \sigma(s)\sigma(p)\frac{\partial \dot{s}}{\partial p} + \sigma(c)\sigma(p)\frac{\partial \dot{c}}{\partial p} + \sigma^2(p)\frac{\partial \dot{p}}{\partial p})\,|\dot{x}|\end{aligned}$$

We have: $\frac{\partial \dot{s}}{\partial s} = -\frac{\partial \dot{c}}{\partial s} - \frac{\partial \dot{p}}{\partial s}$ ($\frac{\partial \dot{s}}{\partial s} \leq 0, \frac{\partial \dot{c}}{\partial s} \geq 0, \frac{\partial \dot{p}}{\partial s} \geq 0$), $\frac{\partial \dot{c}}{\partial c} = -\frac{\partial \dot{s}}{\partial c} - \frac{\partial \dot{p}}{\partial c}$ ($\frac{\partial \dot{c}}{\partial c} \leq 0, \frac{\partial \dot{s}}{\partial c} \geq 0, \frac{\partial \dot{p}}{\partial c} \geq 0$), and $\frac{\partial \dot{p}}{\partial p} > -\frac{\partial \dot{s}}{\partial p} - \frac{\partial \dot{c}}{\partial p}$ ($\frac{\partial \dot{p}}{\partial p} \leq 0, \frac{\partial \dot{s}}{\partial p} \geq 0, \frac{\partial \dot{c}}{\partial p} \geq 0$).

Therefore,

$$\begin{aligned}\dot{v}(s,c,p) = ((\sigma(c)\sigma(s)-1)\frac{\partial \dot{c}}{\partial s} + (\sigma(p)\sigma(s)-1)\frac{\partial \dot{p}}{\partial s} \quad (\sigma(s)\sigma(c)-1)\frac{\partial \dot{s}}{\partial c} + (\sigma(p)\sigma(c)-1)\frac{\partial \dot{p}}{\partial c} \\ \sigma(s)\sigma(p)\frac{\partial \dot{s}}{\partial p} + \sigma(c)\sigma(p)\frac{\partial \dot{c}}{\partial p} + \frac{\partial \dot{p}}{\partial p})\,|\dot{x}|\end{aligned} \tag{2.3.5}$$

Remind that $\{\sigma(s), \sigma(c), \sigma(p)\} \in \{1, -1\}$ and we have $\frac{\partial \dot{p}}{\partial p} > -\frac{\partial \dot{s}}{\partial p} - \frac{\partial \dot{c}}{\partial p}$, then:

- $\forall \{\sigma(s), \sigma(c), \sigma(p)\}$, then $\frac{\partial \dot{p}}{\partial p} + \sigma(s)\sigma(p)\frac{\partial \dot{s}}{\partial p} + \sigma(c)\sigma(p)\frac{\partial \dot{c}}{\partial p}$ is strictly negative.

Moreover if $\dot{p} = 0$, we have $\sigma(p) = 0$, and therefore:

- $(\sigma(c)\sigma(s)-1)\frac{\partial \dot{c}}{\partial s} + (\sigma(p)\sigma(s)-1)\frac{\partial \dot{p}}{\partial s} < 0$.
- $(\sigma(s)\sigma(c)-1)\frac{\partial \dot{s}}{\partial c} + (\sigma(p)\sigma(c)-1)\frac{\partial \dot{p}}{\partial c} < 0$.

Therefore, $\forall x \neq (s^*, c^*, p^*) : \dot{v}(s,c,p) < 0$. We have also:

$$\begin{aligned}v(s,c,p) &= |\dot{s}| + |\dot{c}| + |\dot{p}| \\ &= |u - k_1 s(E_0 - c) + k_{-1}c| + |k_1 s(E_0 - c) - k_{-1}c - k_2 c + k_{-2}p(E_0 - c)| \\ &\quad + |k_2 c - k_{-2}p(E_0 - c)|\,.\end{aligned} \tag{2.3.6}$$

So, if $\sqrt{s^2 + c^2 + p^2} \to +\infty$ then $v(s,c,p) = |\dot{s}| + |\dot{c}| + |\dot{p}| \to +\infty$. Therefore the unique equilibrium $x = (s^*, c^*, p^*)$ of the open system is globally asymptotically stable. □

2.3.2.2 Global stability using monotone systems theory

We proved using a Lyapunov function (see above) that the equilibrium (if it exist, $u < k_2 E_0$), is globally asymptotically stable. We want now to show the stability with another method, with a basin of attraction as large as possible with fulfills the constraints on the variables (they are nonnegative and $c < E_0$). To do so, we use a simple property of cooperative (or monotone) systems. The theory of monotone systems has strong links with compartmental systems, but it is more general. The basic facts and the theorem we need are recalled in the appendix.

Proposition 2.8. *If $u < k_2 E_0$, then the equilibrium exists ; it is globally stable in the invariant hyperrectangle defined by $(0, 0, 0)$ as the lower corner, and (s, c, p) as the upper corner, with $e = E_0 - c$ very small, and*

$$p = \frac{k_2 E_0 - u - k_2 e}{k_{-2} e}$$

$$s = \frac{k_{-1} E_0 - k_{-1} e + u}{k_1 e}$$

The values of p and s tends to infinity when c tends to E_0.

The theorem says that if the equilibrium exists, it is globally stable in nearly all the possible space.

Proof. Monotone systems conserve the partial ordering of two solutions; if there is a point greater than the equilibrium point (with respect to the usual partial ordering) where all the derivatives are nonpositive, then the trajectory issued from this point is always decreasing, and converges toward the equilibrium point. Similarly, if there is a point smaller than the equilibrium where the derivatives are nonnegative, then the solution from this point increases until the equilibrium. The whole hyperrectangle built with these two points is invariant, and all the trajectories initiating in this rectangle converge toward the equilibrium.

Thus we have to find the two extreme points vertices of the rectangle. For the lower one, it is easy ; we choose $s = c = p = 0$ and obtain that the vector field is

$$\dot{s} = u; \dot{c} = 0; \dot{p} = 0$$

The vector field is nonnegative.

The case of the upper vertex is more intricate: we want to find a point (s, c, p) as large as possible, such that the vector field is nonpositive. If $c = E_0$, it does not work because

$\dot{s} = u + k_{-1}E_0 > 0$. Thus we have to choose a c a bit smaller than E_0. Remember that $e = E_0 - c$. We write the inequality we wish to fulfill:

$$\begin{aligned} &u - k_1 s(E_0 - c) + k_{-1}c < 0 \\ &k_1 s(E_0 - c) - k_{-1}c - k_2 c + k_{-2}p(E_0 - c) < 0 \\ &k_2 c - k_{-2}p(E_0 - c) - kp < 0 \end{aligned} \tag{2.3.7}$$

Remark that if we fix $e = E_0 - c$, the system becomes linear with respect to the two other variables s and p. Summing the two first inequalities and replacing c by $E_0 - e$, we obtain:

$$k_{-2}pe < k_2 E_0 - u - k_2 e \tag{2.3.8}$$

But we know (because of the equilibrium existence) that $k_2E_0 - u > 0$, therefore we can choose e very small such that $k_2E_0 - u - k_2e > 0$. Then we can choose

$$p = \frac{k_2E_0 - u - k_2e}{k_{-2}e} \tag{2.3.9}$$

The second inequality of the system is transformed with the help of (2.3.8):

$$k_1 se - k_{-1}E_0 + k_{-1}e - k_2E_0 + k_2e + k_{-2}pe <$$

$$k_1 se - k_{-1}E_0 + k_{-1}e - u$$

and is fulfilled if

$$s = \frac{k_{-1}E_0 - k_{-1}e + u}{k_1 e} \tag{2.3.10}$$

We remark that when $e \longrightarrow 0$ in the equations (2.3.9) (2.3.10), then the values of s and p tends to infinity. That means that the upper point can be chosen such that c is very close to E_0, and the values of s and p will be very large. We remark that local stability is obtained by the same way.

In conclusion we obtain the global stability in the hyperrectangle with the upper and lower vertives given above, see figure 2.3.3.

□

Remark 2.9. If we consider the open irreversible Michaelis-Menten system, as previously, we can prove that there exists a positive equilibrium (s^*, c^*, p^*) (with $c^* < E_0$) if and only if $u < k_2E_0$. It is easy to check that the Jacobian matrix of the system is still compartmental thus we can still apply the proposition in appendix A.1 to study the

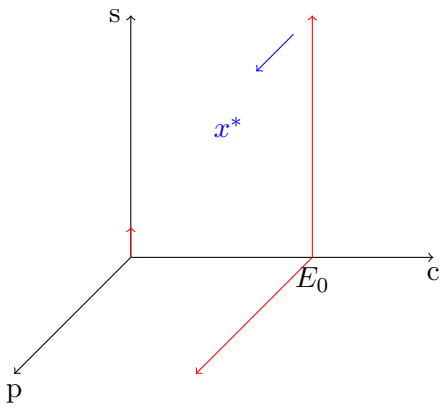

FIGURE 2.3.3: The vector fields in the upper and lower vertives of the hyperrectangle.

stability of the equilibrium (in the case $u < k_2 E_0$). Therefore we obtain the global stability in the hyperrectangle defined by $(0,0,0)$ as the lower corner, and (s,c,p) as the upper corner, with $c > c^*$ such that $e = E_0 - c$ is very small, and p as large as desired, and $s = \frac{u+k_{-1}(E_0-e)}{k_1 e}$. Here also s tends to infinity when c tends to E_0.

2.3.3 The switch between two regimes: the equilibrium exists and do not exist

To see what happen if there is a switch between these two regimes (equilibrium exists or not), we chose a varying input $u(t)$, having two values corresponding to the existence, or not, of an equilibrium. By varying the frequency of commutation between the two inputs, we may obtain a limit cycle which is globally stable (see Fig.2.3.4). The solution could also becomes unbounded (see Fig.2.3.5) in the case that the time interval when there is no equilibrium is longer than the time interval when there is equilibrium.

These results are obtained by numerical simulations, but we think that some proofs are possible.

2.4 The closed enzymatic chain

In the following we aim to apply these tools to a metabolic chain. Each step of the chain is a reversible enzymatic reaction, and first without input and output. The diagram of the chain with $n-1$ enzymatic reactions is as follows:

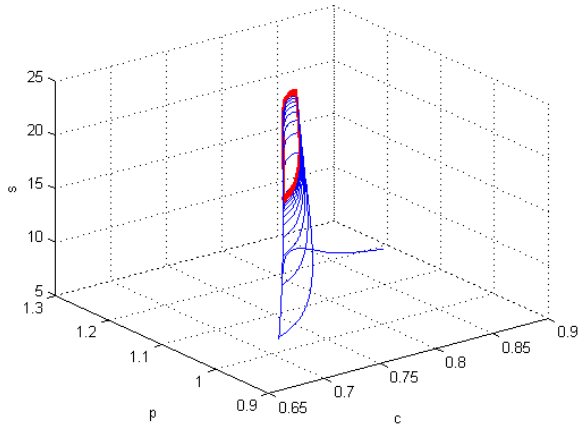

FIGURE 2.3.4: Convergence of a trajectory toward a limit cycle (red) in the state space, with an input u commuting between $u_1 = 0.2$ (equilibrium exists) during $t_1 = 40$ and $u_2 = 0.8$ (no equilibrium) during $t_2 = 15$. Other parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.5, E_0 = 1, k = 0.3, s(t_0) = 12, c(t_0) = 0.8, p(t_0) = 1$.

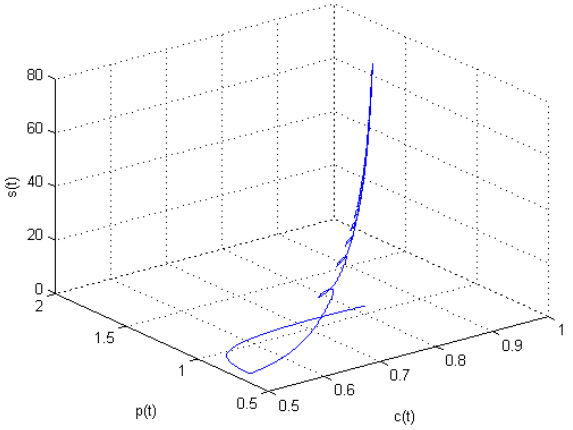

FIGURE 2.3.5: Simulation with an input u commuting between $u > k_2E_0 = 0.8$ during $t_1 = 15$ and $u < k_2E_0 = 0.2$, during $t_2 = t_1 = 15$

$$\underset{+\,E_1}{S_1} \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} C_1 \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} \underset{+\,E_1}{S_2} + E_2 \underset{k_{-21}}{\overset{k_{21}}{\rightleftharpoons}} C_2 \underset{k_{-22}}{\overset{k_{22}}{\rightleftharpoons}} \underset{+\,E_2}{S_3}...S_i + E_i \underset{k_{-i1}}{\overset{k_{i1}}{\rightleftharpoons}} C_i \underset{k_{-i2}}{\overset{k_{i2}}{\rightleftharpoons}} \underset{+\,E_i}{S_{i+1}}... \underset{k_{-(n-1)2}}{\overset{k_{(n-1)2}}{\rightleftharpoons}} \underset{+\,E_{n-1}}{S_n}$$

Therefore we get:

$$\begin{cases} \dot{s_1} = -k_{11}s_1(E_{01} - c_1) + k_{-11}c_1 \\ \dot{c_1} = k_{11}s_1(E_{01} - c_1) - k_{-11}c_1 - k_{12}c_1 + k_{-12}s_2(E_{01} - c_1) \\ \dot{s_2} = k_{12}c_1 - k_{-12}s_2(E_{01} - c_1) - k_{21}s_2(E_{02} - c_2) + k_{-21}c_2 \\ \dot{c_2} = k_{21}s_2(E_{02} - c_2) - k_{-21}c_2 - k_{22}c_2 + k_{-22}s_3(E_{02} - c_2) \\ \quad \vdots \\ \dot{s_i} = k_{(i-1)2}c_{i-1} - k_{-(i-1)2}s_i(E_{0(i-1)} - c_{i-1}) - k_{i1}s_i(E_{0i} - c_i) + k_{-i1}c_i \\ \dot{c_i} = k_{i1}s_i(E_{0i} - c_i) - k_{-i1}c_i - k_{i2}c_i + k_{-i2}s_{i+1}(E_{0i} - c_i) \\ \quad \vdots \\ \dot{s_n} = k_{(n-1)2}c_{n-1} - k_{-(n-1)2}s_n(E_{0(n-1)} - c_{n-1}) \end{cases} \tag{2.4.1}$$

Remark that at the equilibrium we have for all $i = 1, .., n-1$:

$$\begin{cases} -k_{i1}s_i^*(E_{0i} - c_i^*) + k_{-i1}c_i^* = 0 \\ -k_{i2}c_i^* + k_{-i2}s_{i+1}^*(E_{0i} - c_i^*) = 0 \end{cases} \tag{2.4.2}$$

$$\Rightarrow \begin{cases} k_{i1}s_i^*(E_{0i} - c_i^*) = k_{-i1}c_i^* \\ k_{-i2}s_{i+1}^*(E_{0i} - c_i^*) = k_{i2}c_i^* \end{cases} \tag{2.4.3}$$

where :

$$\Rightarrow \begin{cases} s_i^* = \dfrac{k_{-i1}c_i^*}{k_{i1}(E_{0i} - c_i^*)} \\ c_i^* = \dfrac{1}{k_{i2}}k_{-i2}s_{i+1}^*(E_{0i} - c_i^*) \end{cases} \tag{2.4.4}$$

The Jacobian matrix $J(s_1, c_1, s_2, c_2, ..., s_i, c_i, ..., s_n)$ is :

$$\begin{pmatrix} -k_{11}(E_{01}-c_1) & k_{11}s_1+k_{-11} & 0 & \dots \\ k_{11}(E_{01}-c_1) & -k_{11}s_1-k_{-11}-k_{12}-k_{-12}s_2 & k_{-12}(E_{01}-c_1) & \dots \\ 0 & k_{12}+k_{-12}s_2 & -k_{-12}(E_{01}-c_1)-k_{21}(E_{02}-c_2) & \dots \\ 0 & 0 & k_{21}(E_{02}-c_2) & \dots \\ 0 & 0 & 0 & \dots \\ \vdots & \vdots & \vdots & \ddots \\ 0 & 0 & 0 & \dots \end{pmatrix}$$

Remark 2.10. The study of a closed chain of $n-1$ such enzymatic reactions is possible with the same tools and the same results.

The system is closed, in the sense that $\dot{s}_1+\dot{c}_1+\dot{s}_2+\dot{c}_2+.....+\dot{s}_n=0$. Moreover, the Jacobian matrix is compartmental.

We can draw the graph of $J(s_1,c_1,s_2,c_2,...,s_i,c_i,...,s_n)$ and check that it is strongly connected (under the assumption that all parameters are strictly positives). Then this theorem applies:

Theorem 2.11. *Let $M(s_1,c_1,s_2,c_2,...,s_i,c_i,...,s_n)=s_1+c_1+s_2+c_2+...+s_i+c_i+...+s_n$ the (fixed) total concentration of the closed system. The Jacobian matrix of the system is irreducible (the system is strongly connected) and compartmental; then for any $M_0>0$, hyperplane $H_0=\{s_1,c_1,s_2,c_2,...,s_i,c_i,...,s_n)\in\Re^n_+ : M(s_1,c_1,s_2,c_2,...,s_i,c_i,...,s_n)=M_0>0\}$ is forward invariant and contains a unique globally stable equilibrium in H_0.*

2.5 Open enzymatic chain with one input and output

We consider that we have one fixed input on the first metabolite, and one output from the last. To simplify the computations we take only two enzymatic reactions but the results are the same for general $(n-1)$ enzymatic reactions to produce S_n. The reaction scheme of the chain is as follows :

$$\xrightarrow{u} S_1+E_1 \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} C_1 \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} \underset{+\,E_1}{S_2+E_2} \underset{k_{-21}}{\overset{k_{21}}{\rightleftharpoons}} C_2 \underset{k_{-22}}{\overset{k_{22}}{\rightleftharpoons}} E_2+S_3 \xrightarrow{k}$$

As before, we use the conservation laws to set $e_1=(E_{01}-c_1)$ and $e_2=(E_{02}-c_2)$ and obtain:

$$\begin{cases} \dot{s_1} = u - k_{11}s_1(E_{01} - c_1) + k_{-11}c_1 \\ \dot{c_1} = k_{11}s_1(E_{01} - c_1) - k_{-11}c_1 - k_{12}c_1 + k_{-12}s_2(E_{01} - c_1) \\ \dot{s_2} = k_{12}c_1 - k_{-12}s_2(E_{01} - c_1) - k_{21}s_2(E_{02} - c_2) + k_{-21}c_2 \\ \dot{c_2} = k_{21}s_2(E_{02} - c_2) - k_{-21}c_2 - k_{22}c_2 + k_{-22}s_3(E_{02} - c_2) \\ \dot{s_3} = k_{22}c_2 - k_{-22}s_3(E_{02} - c_2) - ks_3 \end{cases} \tag{2.5.1}$$

Proposition 2.12. *There exists a positive unique equilibrium* $(s_1^*, c_1^*, s_2^*, c_2^*, s_3^*)$ *if and only if* $u < k_{i2}E_{0i}$ *for* $i = 1, 2$.

Proof. Note that at the equilibrium we have for $i = 1, 2$:

$$\begin{cases} u - k_{i1}s_i^*(E_{0i} - c_i^*) + k_{-i1}c_i^* = 0 \\ u - k_{i2}c_i^* + k_{-i2}s_{i+1}^*(E_{0i} - c_i^*) = 0 \quad c_i^* < E_{0i} \end{cases} \tag{2.5.2}$$

and

$$u = ks_3^* \tag{2.5.3}$$

Therefore, for $i = 1, 2$:

$$\begin{cases} s_i^* = \dfrac{u + k_{-i1}c_i^*}{k_{i1}(E_{0i} - c_i^*)} \\ c_i^* = \dfrac{u + k_{-i2}s_{i+1}^*E_{0i}}{k_{-i2}s_{i+1}^* + k_{i2}} \end{cases} \tag{2.5.4}$$

and

$$s_3^* = \frac{u}{k}$$

$$c_2^* = \frac{u + k_{-22}s_3^*E_{02}}{k_{-22}s_3^* + k_{22}} = \frac{(1 + \frac{k_{-22}E_{02}}{k})u}{\frac{k_{-22}}{k}u + k_{22}}$$

This function of u is strictly increasing from 0 (for $u = 0$) to $E_{02} + \frac{k}{k_{-22}}$ when u tends to infinity. The constraint $c_2^* < E_{02}$ gives $\frac{(1+\frac{k_{-22}E_{02}}{k})u}{\frac{k_{-22}}{k}u+k_{22}} < E_{02}$ which implies $u < k_{22}E_{02}$. If this inequality is fulfilled, we can compute the value of $s_2^* = \frac{u+k_{-21}c_2^*(u)}{k_{21}(E_{02}-c_2^*(u))}$.

Similarly we can also compute c_1^* from (2.5.4) with the value of $s_2^*(u)$ from above. Remember that $c_2^*(u) = 0$ if $u = 0$, therefore $c_1^*(0) = 0$. Moreover the function $c_1^*(u)$ is increasing as a composite function of increasing functions. We also have the constraint

$c_1^* < E_{01}$, so as previously we conclude from expression of c_1^* in (2.5.4) that $u < k_{12}E_{01}$, which ends the proof. □

In the following the aim is to study the global stability of the equilibrium when $u < k_{12}E_{01}$, and $u < k_{22}E_{02}$, i.e. the case where the equilibrium exists.

Proposition 2.13. *If for $i = 1, 2$: $u < k_{i2}E_{0i}$, then the equilibrium exists and is globally stable in the invariant hyperrectangle defined by $(0, 0, 0, 0, 0)$ as the lower corner, and $(s_1, c_1, s_2, c_2, s_3)$ as the upper corner, such that : $c_1 = E_{01} - e_1$, $c_2 = E_{02} - e_2$ with e_1, e_2 small enough to satisfy:*

1. $k_{12}(E_{01} - e_1) - u > 0$
2. $k_{22}(E_{02} - e_2) - u > 0$

The coordinates of this upper corner point are:

$$e_2 = \frac{(u + k_{-21}E_{02})k_{-12}e_1}{k_{21}(k_{12}(E_{01} - e_1) - u) + k_{-21}k_{-12}e_1}$$

$s_1 = \frac{u+k_{-11}(E_{01}-e_1)}{k_{11}e_1}$; $s_2 = \frac{k_{12}(E_{01}-e_1)-u}{k_{-12}e_1} = \frac{u+k_{-21}(E_{02}-e_2)}{k_{21}e_2}$; $s_3 = \frac{k_{22}(E_{02}-e_2)-u}{k_{-22}e_2}$.

Note that s_1 , s_2 , s_3 tend to infinity when e_1 tends to 0 and e_2 tends to 0. Moreover, the equilibrium is locally exponentially asymptotically stable.

Proof. The Jacobian matrix of system (2.5.1) is still compartmental so we can also investigate the stability of the equilibrium when $u < k_{12}E_{01}$ and $u < k_{22}E_{02}$ (which are the conditions for existence of equilibrium) by using the proposition (A.1) of monotone systems (cf. appendix). If there is a point greater than the equilibrium point (with respect to the usual partial ordering) where all the derivatives are nonpositive and similarly, if there is a point smaller than the equilibrium where the derivatives are nonnegative, then the whole hyperrectangle built with these two points is invariant, and all the trajectories initiating in this rectangle converge toward the equilibrium.

Thus we have to find the two extreme vertices of the rectangle. For the lower one, it is easy ; we choose $s_1 = c_1 = s_2 = c_2 = s_3 = 0$ and obtain that the following vector field

$$\dot{s}_1 = u; \dot{c}_1 = 0; \dot{s}_2 = 0; \dot{c}_2 = 0; \dot{s}_3 = 0$$

which is nonnegative.

For the upper vertex, we write the inequality we wish to fulfill:

$$\begin{aligned}
&u - k_{11}s_1(E_{01} - c_1) + k_{-11}c_1 \leq 0\\
&k_{11}s_1(E_{01} - c_1) - k_{-11}c_1 - k_{12}c_1 + k_{-12}s_2(E_{01} - c_1) \leq 0\\
&k_{12}c_1 - k_{-12}s_2(E_{01} - c_1) - k_{21}s_2(E_{02} - c_2) + k_{-21}c_2 \leq 0\\
&k_{21}s_2(E_{02} - c_2) - k_{-21}c_2 - k_{22}c_2 + k_{-22}s_3(E_{02} - c_2) \leq 0\\
&k_{22}c_2 - k_{-22}s_3(E_{02} - c_2) - ks_3 \leq 0
\end{aligned} \tag{2.5.5}$$

Firstly, to obtain a basin of attraction as large as possible we fixed c_1 a little bit smaller than E_{01} ($c_1 = E_{01} - e_1$) and c_2 a little bit smaller than E_{02} ($c_2 = E_{02} - e_2$). We firstly study necessary conditions. Summing the first four inequalities and taking $c_2 = E_{02} - e_2$, we obtain :

$$u - k_{22}(E_{02} - e_2) + k_{-22}s_3(E_{02} - c_2) \leq 0 \Rightarrow s_3 \leq \frac{k_{22}(E_{02} - e_2) - u}{k_{-22}e_2}. \tag{2.5.6}$$

We know that $k_{22}E_{02} - u > 0$ because the equilibrium exists, therefore we can choose $s_3 = \frac{k_{22}(E_{02}-e_2)-u}{k_{-22}e_2}$ with e_2 small enough to fulfill $k_{22}E_{02} - u - k_{22}e_2 > 0$.

Similarly summing the first three inequalities and taking $c_2 = E_{02} - e_2$ we have :

$$u - k_{21}s_2e_2 + k_{-21}(E_{02} - e_2) \leq 0 \Rightarrow s_2 \geq \frac{u + k_{-21}(E_{02} - e_2)}{k_{21}e_2} \tag{2.5.7}$$

then we can choose :

$$s_2 = \frac{u + k_{-21}(E_{02} - e_2)}{k_{21}e_2}. \tag{2.5.8}$$

Summing also the first two inequalities and taking $c_1 = E_{01} - e_1$ we have :

$$u - k_{12}(E_{01} - e_1) + k_{-12}s_2e_1 \leq 0 \Rightarrow s_2 \leq \frac{k_{12}(E_{01} - e_1) - u}{k_{-12}e_1} \tag{2.5.9}$$

Then we can choose :

$$s_2 = \frac{k_{12}(E_{01} - e_1) - u}{k_{-12}e_1}; \tag{2.5.10}$$

We also know that $k_2E_0 - u > 0$, thus we take e_1 small enough to have $k_{12}(E_{01} - e_1) - u > 0$. From the equations (2.5.8) and (2.5.10) we can write :

$$s_2 = \frac{k_{12}(E_{01} - e_1) - u}{k_{-12}e_1} = \frac{u + k_{-21}(E_{02} - e_2)}{k_{21}e_2}.$$

therefore we can compute e_2 as a function of e_1

$$e_2 = \frac{(u + k_{-21}E_{02})k_{-12}e_1}{k_{21}(k_{12}(E_{01} - e_1) - u) + k_{-21}k_{-12}e_1} \tag{2.5.11}$$

It is easy to check with this equation that e_2 tends to 0 when e_1 tends to zero. Therefore it is possible to choose e_1 and e_2 small together to check the constraints 1. and 2. of the theorem.

Then we can choose:

$s_1 = \frac{u+k_{-11}(E_{01}-e_1)}{k_{11}e_1}$;

$s_2 = \frac{k_{12}(E_{01}-e_1)-u}{k_{-12}e_1} = \frac{u+k_{-21}(E_{02}-e_2)}{k_{21}e_2}$;

$s_3 = \frac{k_{22}(E_{02}-e_2)-u}{k_{-22}e_2}$.

Note that when e_1 tends to 0 and e_2 tends to 0, s_1, s_2, and s_3 tends ot infinity; we obtain the following vector field $\dot{s_1} = 0$, $\dot{c_1} = 0$, $\dot{s_2} = 0$,$\dot{c_2} = 0$ and $\dot{s_3} \to -\infty$. This vector field is nonpositive, as desired.

In conclusion we obtain the global stability in the hyperrectangle with the upper and lower vertices given above.

Concerning the exponential stability, it is easy to check that the Jacobian matrix around the equilibrium has a graph which is outflow-connected (see appendix), and therefore the matrix is bijective and asymptotically stable. □

We next performed simulations for the case when the equilibrium exists ($u < k_{12}E_{01}$, $u < k_{22}E_{02}$) and a case when the equilibrium does not exist ($k_{12}E_{01} < u < k_{22}E_{02}$). The results of simulations are shown in the figures (2.5.1) and (2.5.2). As predicted, in the first case, the system is stable and converges to the equilibrium, whereas, in the second case, it is not stable and the concentration of first substrate S_1 grows ot infinity.

2.6 Coupling metabolic and genetic systems

Until now we have studied the models where the total concentration of the enzymes remained constant. This is not very realistic: in fact the enzymes are made by the cellular global machinery. Moreover the metabolic enzymatic chain reactions are biochemical reactions whose final product can influence the production of enzymes via gene regulation. These cross interactions between genetic and metabolic systems form a coupled system controlled by feedback loops [32]. Let's consider a coupled metabolic genetic system defined by the following equations :

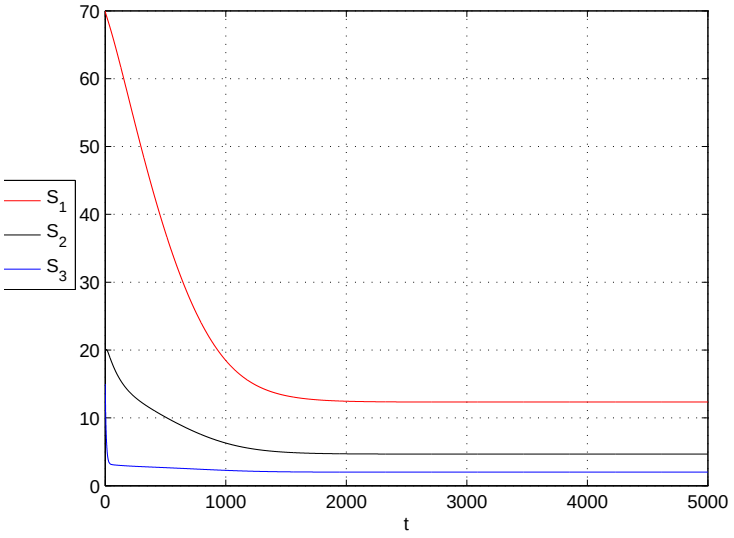

FIGURE 2.5.1: Simulation of system (2.5.1) for $u < k_{12}E_{01}$ and $u < k_{22}E_{02}$, with the following parameters values $k_{11} = 0.2, k_{-11} = 0.2, k_{12} = 0.4, k_{-12} = 0.2, k_{21} = 0.3, k{-}21 = 0.2, k_{22} = 0.4, k_{-22} = 0.2, k = 0.1, E_{01} = 1, E_{02} = 1, u = 0.2, S_{01} = 70, S_{02} = 0.8, S_{03} = 20, S_{04} = 0.7, S_{05} = 15$. The trajectory converges toward the equilibrium.

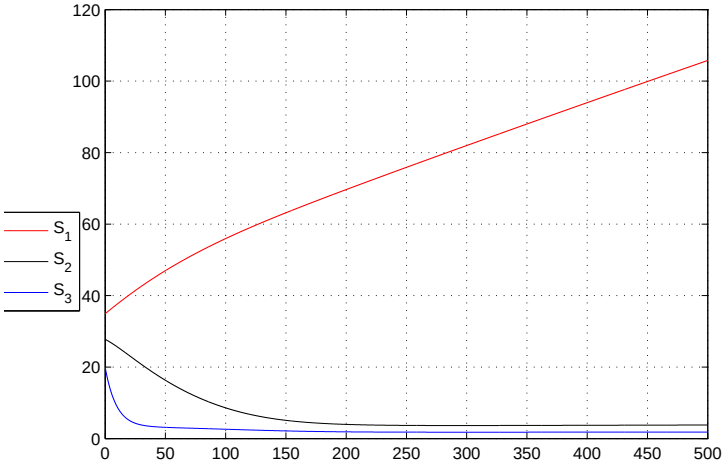

FIGURE 2.5.2: Simulation of system (2.5.1) for $k_{12}E_{01} < u < k_{22}E_{02}$, with the following parameters values $k_{11} = 0.2, k_{-11} = 0.2, k_{12} = 0.2, k_{-12} = 0.2, k_{21} = 0.3, k{-}21 = 0.2, k_{22} = 0.4, k_{-22} = 0.2, k = 0.1, E_{01} = 1, E_{02} = 1, u = 0.3, S_{01} = 35, S_{02} = 0.8, S_{03} = 28, S_{04} = 0.7, S_{05} = 20$. There is no equilibrium and substrate s_1 grows to infinity.

$$\dot{s} = f(s, e)\ ;\ \ \dot{e} = g(s, e) \tag{2.6.1}$$

where s is the vector of metabolite concentrations, e is the vector of enzyme concentrations (see Fig.2.6.1).

For example, if an end-product s_n of the metabolic chain represses the expression of the gene associated to the enzyme e_1, the equation could classically be:

$$\dot{e}_1 = \alpha h^-(s_n) - \gamma e_1$$

where the first term represents the repression (h^- is a decreasing Hill function) and the second term is a linear unregulated degradation.

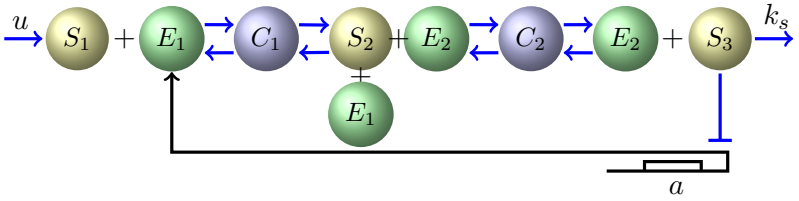

FIGURE 2.6.1: A metabolic chain with enzymatic regulation; the product of the chain represses the expression of the enzyme catalyzing the first step of the chain

The dynamics of the metabolic system is very fast compared to the genetic system. If the metabolic system is globally stable, it will go very quickly to the equilibrium point s^*; under appropriate conditions which are known as Tikhonov's Theorem we can apply the quasi-steady state approximations leading to $s^* = \varphi(e)$. The genetic system becomes :

$$\dot{e} = g(\varphi(e), e)$$

Importantly this times-scale reduction is valid only if the metabolic system is globally stable, therefore it is necessary to check if the equilibrium of the metabolic system exists and if it is globally stable. To clarify the conditions for using the quasi-steady state approximations to reduce metabolic-genetic systems, we will recall Tikhonov's Theorem as in [66]; this theorem applies to reduced systems of the form:

$$\begin{aligned} \dot{x} &= f(x, y, \epsilon) \\ \dot{y} &= \frac{1}{\epsilon} g(x, y, \epsilon). \end{aligned} \tag{2.6.2}$$

where $x \in \Re_+^n$, $y \in \Re_+^m$, $0 < \epsilon \ll 1$ (ϵ is a small parameter), and f, g are smooth functions, $x(0) = x_0, y(0) = y_0$.

So, when ϵ tends to 0 ($\dot{y}$ evolves very rapidly compared to $\dot{x}$), the system (2.6.2) is equivalent to the system:

$$\begin{aligned} \dot{x} &= f(x, y, 0) \\ g(x, y, 0) &= 0. \end{aligned}$$

This is valid only if the fast subsystem $\dot{y} = g(x, y, 0)$ satisfies some conditions which are given as follows:

- Existence and uniqueness of the steady state (there exists a unique solution, $y^* = \phi(x)$ of $g(x, y, 0) = 0$).
- Stability of the steady state $y^* = \phi(x)$ (the equilibrium is asymptotically stable and the initial value y_0 belongs to the domain of attraction of the equilibrium $y^* = \phi(x)$, $g(x, y, 0) = 0$).

These conditions are given by Tikhonov's theorem (for a complete description see [40]) and ensure that y will converge rapidly to a *quasi-steady state* ($y = \phi(x)$). Therefore the reduced system using Tikhonov's Theorem is:

$$\dot{x} = f(x, \phi(x), 0), \quad x(0) = x_0.$$

Remark 2.14. According to our previous results, if $u < k_{i2}E_{0i}$ for all $i = 1, .., n-1$, the good hypotheses for the fast subsystems are fulfilled, the metabolic system is stable, and the quasi-steady state reduction can apply.

But if one of the above inequality is not fulfilled, the equilibrium disappears at infinity, and the QSSA reduction maybe become not valid. The enzyme concentration e_i now becomes a dynamical variable, and it may happen that, at the beginning, the equilibrium exists, but disappears after some time because one of the e_i becomes too small.

Before dealing with the coupling metabolic-genetic models, we give the following simple example to show that is is needed to put the system under the good form to reduce it using Tikhonov's Theorem:

Example 2.1. *Consider these reactions schemes :*

$$S_1 \underset{b}{\overset{a}{\rightleftharpoons}} S_2 \quad S_1 + S_2 \xrightarrow{\mu} S_3, \tag{2.6.3}$$

We suppose that the first reaction (reversible) is fast, whereas the second reaction is slow (μ a very small parameter compared to the other parameters a and b). Therefore following the mass action kinetics law we derive system (2.6.4) from the reaction schemes (2.6.3):

$$\begin{cases} \dot{s_1} = -as_1 + bs_2 - \mu s_1 s_2 \\ \dot{s_2} = as_1 - bs_2 - \mu s_1 s_2 \\ \dot{s_3} = \mu s_1 s_2 \end{cases} \tag{2.6.4}$$

where, s_1, s_2, s_3 represent the concentrations of S_1,S_2,S_3 respectively. The form of this system (2.6.4) ($s_4 = (s_1, s_2, s_3)$; $\dot{s_4} = h(x, \mu)$, where $s_4 \in \Re^3$) is different of the form in (2.6.2); the slow part here is coupled with the fast part, and we need to separate them. Therefore first the main problem to reduce this system and for the application of Tikhonov's Theorem is to transform system (2.6.4) to the normal form called Tikhonov form (see system (2.6.2)):

To separate the fast and the slow part we will introduce a new variable $s = s_1 + s_2$ (s willbe a slow variable), therefore the system (2.6.4) becomes:

$$\begin{cases} \dot{s_1} = -as_1 + b(s - s_1) - \mu s_1 (s - s_1) \\ \dot{s} = -2\mu s_1 (s - s_1) \\ \dot{s_3} = \mu s_1 (s - s_1) \end{cases} \tag{2.6.5}$$

which is now of the form of Tikhonov, where s_1 changes fast whereas s and, s_3 change slowly; then for μ small this system (2.6.5) is equivalent to the following system:

$$\begin{cases} \dot{s_1} = -as_1 + b(s - s_1) = 0 \\ \dot{s} = -2\mu s_1 (s - s_1) \\ \dot{s_3} = \mu s_1 (s - s_1) \end{cases} \tag{2.6.6}$$

we need to check that the Jacobian matrix:

$$\frac{\partial(-(a+b)s_1 + bs)}{\partial s_1}$$

is Hurwitz or stable (all the eigenvalues have negative real parts); this means that the slow surface $-(a+b)s_1 + bs = 0$ is attractive. Therefore, the reduced system is given by:

$$\begin{cases} \dot{s} = -2\mu s_1^*(s)(s - s_1^*(s)) = -2\mu \dfrac{ba}{(a+b)^2} s^2 \\ \dot{s_3} = \mu s_1^*(s)(s - s_1^*(s)) = \mu \dfrac{ba}{(a+b)^2} s^2 \end{cases} \tag{2.6.7}$$

In this example it was easy to obtain the fast variable as a function of the slow variable. Usually this is not the case when the fast part is of a high dimension. It will be our case in the next paragraph, therefore we explain our method for this simple example. Frequently we have the existence of first integrals with the fast part of a model of genetic networks. For the reduced system above the fast subsystem is as follows:

$$\begin{pmatrix} \dot{s_1} \\ \dot{s_2} \end{pmatrix} = \begin{pmatrix} -as_1 + bs_2 \\ as_1 - bs_2 \end{pmatrix} \tag{2.6.8}$$

We can notice here that $\dot{s_1} + \dot{s_2} = 0$ *(the system is closed), so the total concentration of the closed system will remain constant. After coupling this subsystem with the slow part it becomes* $\dot{s_1} + \dot{s_2} = \dot{s}$*, and what changes now (slowly) is the total concentration of the closed system. Therefore to prove that the slow surface* $-(a+b)s_1 + bs = 0$ *is attractive, we simply have to prove that the fast subsystem (2.6.8) has a unique globally stable equilibrium* (s_1^*, s_2^*) *with compartmental and monotone techniques.*

2.7 Time-scale reduction of metabolic-genetic systems

In this section we are going to modify the system and suppose that free enzymes are synthesized by genes and also degraded with term γ_e. These terms (synthesis and degradation) will be suppose to be small (slow) with respect to the other terms. As shown above, the metabolites can influence the production of enzymes via gene regulation. In the following we will reduce different coupled metabolic-genetic models using the fact that some variables are faster than others (Time-scale reduction).

2.7.1 Product inhibits the production of enzymes

We consider the full open reversible Michaelis-Menten system, and we consider also that the enzyme E is synthesized by a gene a and that the product P inhibits the production of enzymes via gene regulation, see the following diagram 2.7.1:

In the following we also suppose that if the free form of the enzymes is degraded, then the bound forms are also degraded at the same rate and release the substrates. To clarify, we will give an example:

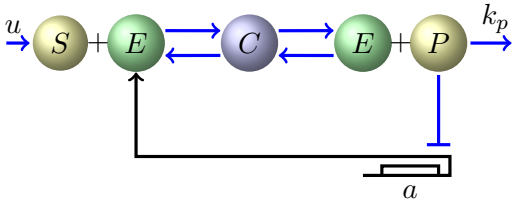

FIGURE 2.7.1: Coupled metabolic genetic system (inhibition of the production of enzymes by the product).

Example 2.2. *Let's take two species A and B, and AB the complex or the bound form of the two species. We suppose also that the behaviour of the concentrations A, B, AB is given by the following system:*

$$\begin{aligned} \dot{a} &= f(a,b) \\ \dot{b} &= g(a,b) \\ \dot{x} &= h(a,b,x) \end{aligned} \tag{2.7.1}$$

where a, b, and x represent respectively the concentrations of the free form of A, B and the concentration of the complex AB of the two species. ($f(a,b), g(a,b)$ and $h(a,b,x)$ are polynomial functions).

So, if we suppose that when the free form of A is degraded, then the bound form of A is also degraded with the same rate, the system becomes:

$$\begin{aligned} \dot{a} &= f(a,b) - k_a a \\ \dot{b} &= g(a,b) \\ \dot{x} &= h(a,b,x) - k_a x \end{aligned} \tag{2.7.2}$$

If we consider that when the bound form of A is degraded, it will release the same quantity of the specie B, the system becomes:

$$\begin{aligned} \dot{a} &= f(a,b) - k_a a \\ \dot{b} &= g(a,b) + k_a x \\ \dot{x} &= h(a,b,x) - k_a x \end{aligned} \tag{2.7.3}$$

This case may be more realistic (when A is degraded, B is not degraded, but it becomes free).

We suppose that the free and the bound form of the enzymes are degraded with the same rate, and release the free substrate. Therefore the dynamics of the model in figure 2.7.1 is:

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 se + k_{-1}c + \gamma_e c \\ \dfrac{de}{dt} = -k_1 se + k_{-1}c + k_2 c - k_{-2}pe + k_e \dfrac{\theta_p^n}{p^n + \theta_p^n} - \gamma_e e, \\ \dfrac{dc}{dt} = k_1 se - k_{-1}c - k_2 c + k_{-2}pe - \gamma_e c, \\ \dfrac{dp}{dt} = k_2 c - k_{-2}pe - kp \end{cases} \tag{2.7.4}$$

red color terms represent the slow dynamical parts.

Because γ_e, and k_e are small with respect to the other parameters, we easily notice that this system has two different scale of time (fast and slow). To transform this system to the form of Tikhonov in (2.6.2), we consider a new variable y, which represents the slow variable, and is the total concentration of the enzyme E.

$$\begin{aligned} & y = e + c \\ & \Rightarrow \dot{y} = \dot{e} + \dot{c} \\ & \Rightarrow \dot{y} = k_e \frac{\theta_p^n}{p^n + \theta_p^n} - \gamma_e y. \end{aligned} \tag{2.7.5}$$

By replacing e with $(y - c)$ the system becomes :

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 s(y - c) + k_{-1}c + \gamma_e c \\ \dfrac{dc}{dt} = k_1 s(y - c) - k_{-1}c - k_2 c + k_{-2}p(y - c) - \gamma_e c \\ \dfrac{dp}{dt} = k_2 c - k_{-2}p(y - c) - kp \\ \dot{y} = k_e \frac{\theta_p^n}{p^n + \theta_p^n} - \gamma_e y \end{cases} \tag{2.7.6}$$

which is in the form of Tikhonov, where the fast system is given by:

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 s(y - c) + k_{-1}c \\ \dfrac{dc}{dt} = k_1 s(y - c) - k_{-1}c - k_2 c + k_{-2}p(y - c) \\ \dfrac{dp}{dt} = k_2 c - k_{-2}p(y - c) - kp \end{cases} \tag{2.7.7}$$

and, the dynamics of the slow system is:

$$\dot{y} = k_e \frac{\theta_p^n}{p^n + \theta_p^n} - \gamma_e y.$$

For the fast subsystem, we can prove as previously (because the fast subsystem is the same as in (2.7.4)) that if $u < k_2 y$, the fast part has an equilibrium which is unique and globally stable. Therefore if $u < k_2 y$ then we can apply Tikhonov theorem. The equilibrium for the fast subsystem is:

$$\begin{aligned} p &= \frac{u}{k} \\ c &= \frac{(k + k_{-2} y)u}{k_2 k + k_{-2} u} \\ s &= \frac{u + k_{-1} c}{k_1 (y - c)}. \end{aligned}$$

Therefore, if we consider p at the equilibrium, the reduced system becomes:

$$\dot{y} = k_e \frac{\theta_p^n}{(\frac{u}{k})^n + \theta_p^n} - \gamma_e y.$$

which is linear. In the following we have performed numerical simulations to show the similarity between the full system and the reduced system (quasi-steady state). The dashed line and the full line show the evolution of the reduced and the complete systems respectively. The results of simulation is given in figures (2.7.2),(2.7.3). In the case that the equilibrium of the fast system exists if $u < k_2 y(t)$, we notice that the approximation is valid and the reduced system is similar to the full system.

For the case that the equilibrium of the fast system disappears at infinity if $u > k_2 y(t)$, see figure (2.7.3), the behaviour of the reduced system and the full system are different, and we can see that the total concentration of the enzyme y of the reduced system deviates from the full system.

Remark 2.15. We notice or conclude that the reduced system (and the reduction procedure) may become not valid if the equilibrium of the fast system does not exist (disappears at infinity).

2.7.2 Product activates the production of enzymes

Consider the same model as before but we suppose in this case that the final product activates the production of enzymes via the gene regulation if it is above a threshold θ_p, see the following diagram (2.7.4):

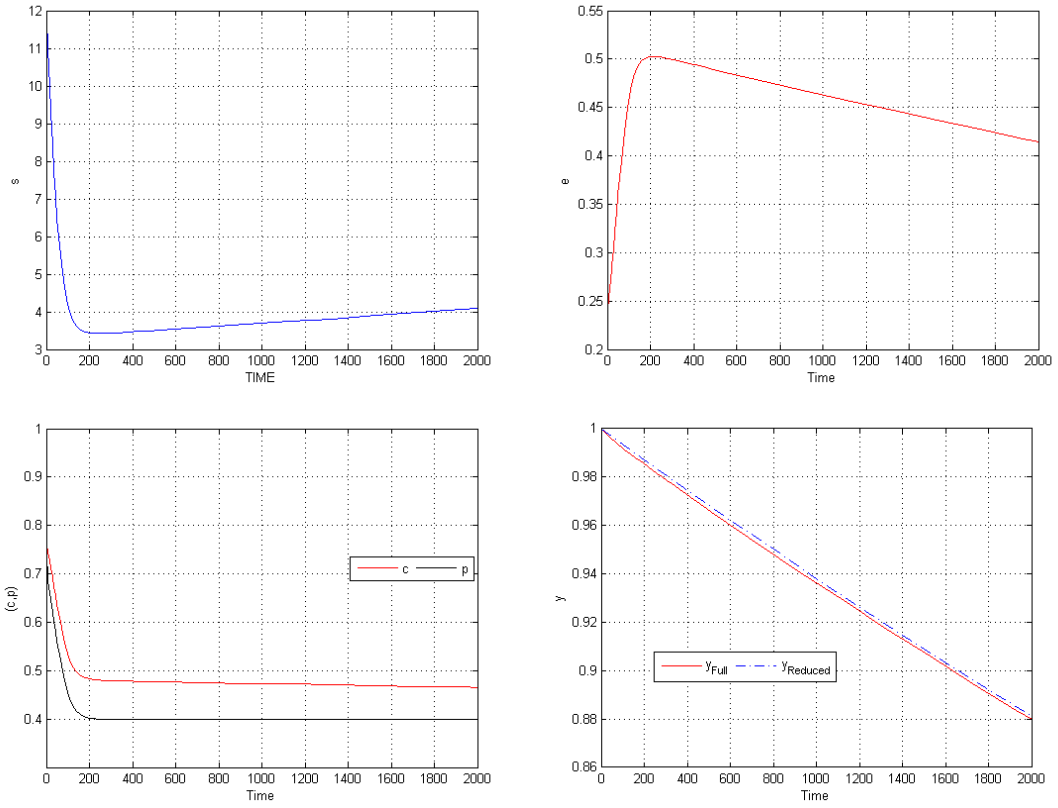

FIGURE 2.7.2: Taking these values of parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.5, k = 0.5, k_e = 0.00005, \gamma_e = 0.00009, \theta_p = 0.4, s(t_0) = 12, e(t_0) = 0.2, c(t_0) = 0.8, p(t_0) = 1, y(t_0) = 1$, $u = 0.2$, we can see that $u < k_2 y(t)$, (the equilibrium of the fast system exists), and the behaviors of the reduced system and the full systems are similar.

The dynamics of this system is given as following:

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 se + k_{-1}c + \gamma_e c \\ \dfrac{de}{dt} = -k_1 se + k_{-1}c + k_2 c - k_{-2}pe + k_e \dfrac{p^n}{p^n + \theta_p^n} - \gamma_e e, \\ \dfrac{dc}{dt} = k_1 se - k_{-1}c - k_2 c + k_{-2}pe - \gamma_e c, \\ \dfrac{dp}{dt} = k_2 c - k_{-2}pe - kp \end{cases} \tag{2.7.8}$$

Taking $y = e + c$ we reduce system 2.7.8, and we get the following system:

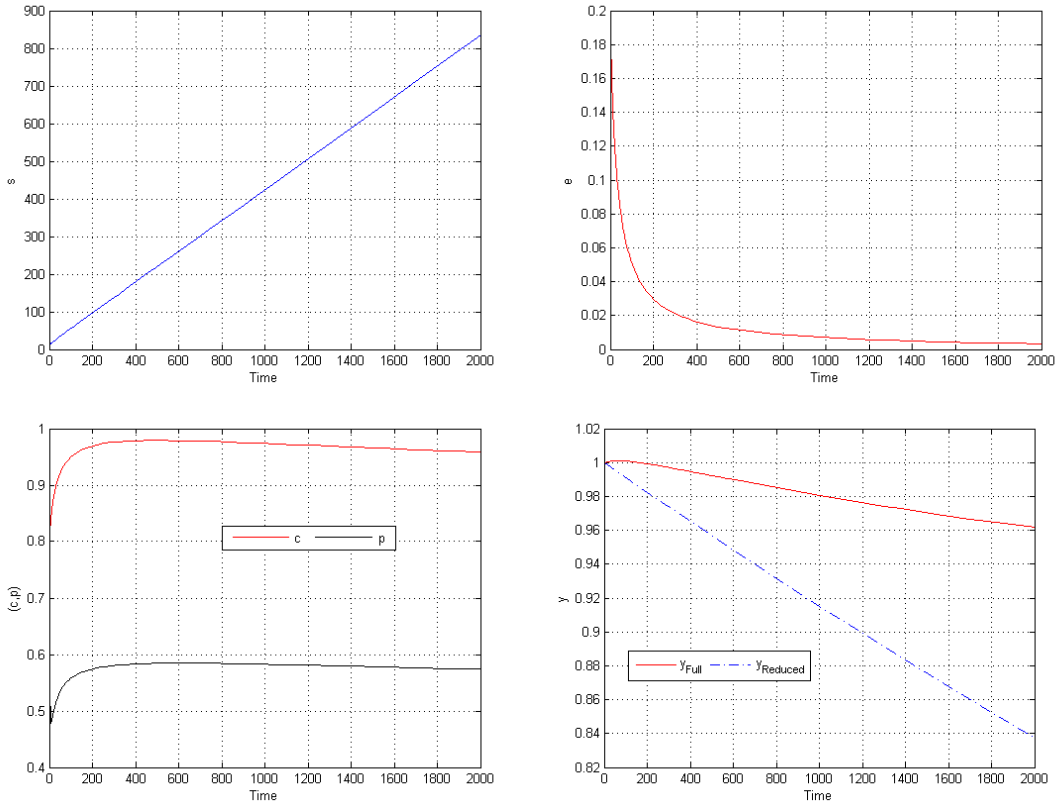

FIGURE 2.7.3: If we take these values of parameters : $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.3, k = 0.5, k_e = 0.0005, \gamma_e = 0.00009, \theta_p = 0.4, s(t_0) = 12, e(t_0) = 0.2, c(t_0) = 0.8, p(t_0) = 1, y(t_0) = 1$, $u = 0.7$, we have $u > k_2 y(t)$ (the equilibrium of the fast system does not exist), the equilibrium of the fast subsystem disappears at infinity and the behaviors of the reduced system and the full systems are completely different.

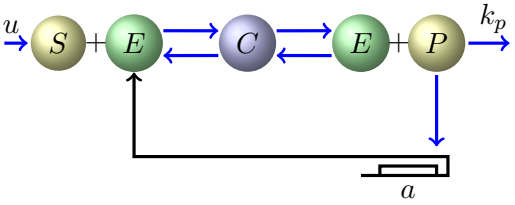

FIGURE 2.7.4: Coupled metabolic genetic system (activation of the production of enzymes by the product).

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 s(y-c) + k_{-1}c + \gamma_e c \\ \dfrac{dc}{dt} = k_1 s(y-c) - k_{-1}c - k_2 c + k_{-2}p(y-c) - \gamma_e c \\ \dfrac{dp}{dt} = k_2 c - k_{-2}p(y-c) - kp \\ \dot{y} = k_e \dfrac{p^n}{p^n + \theta_p^n} - \gamma_e y \end{cases} \tag{2.7.9}$$

For the fast part if $u < k_2 y(t)$, it has an equilibrium which is unique and globally stable. Applying Tikhonov theorem as previously we get the following reduced system:

$$\dot{y} = k_e \frac{(\frac{u}{k})^n}{(\frac{u}{k})^n + \theta_p^n} - \gamma_e y.$$

The results of simulations of the full system and the reduced is given in figures: (2.7.5), (2.7.6): The evolution of the reduced system followed the behavior of the full systems because the equilibrium of the fast system exists. The equilibrium of the fast system may also disappear if we have a strong degradation of the enzyme e and not enough synthesis of the enzyme in the case that $p(t) < \theta_p$.

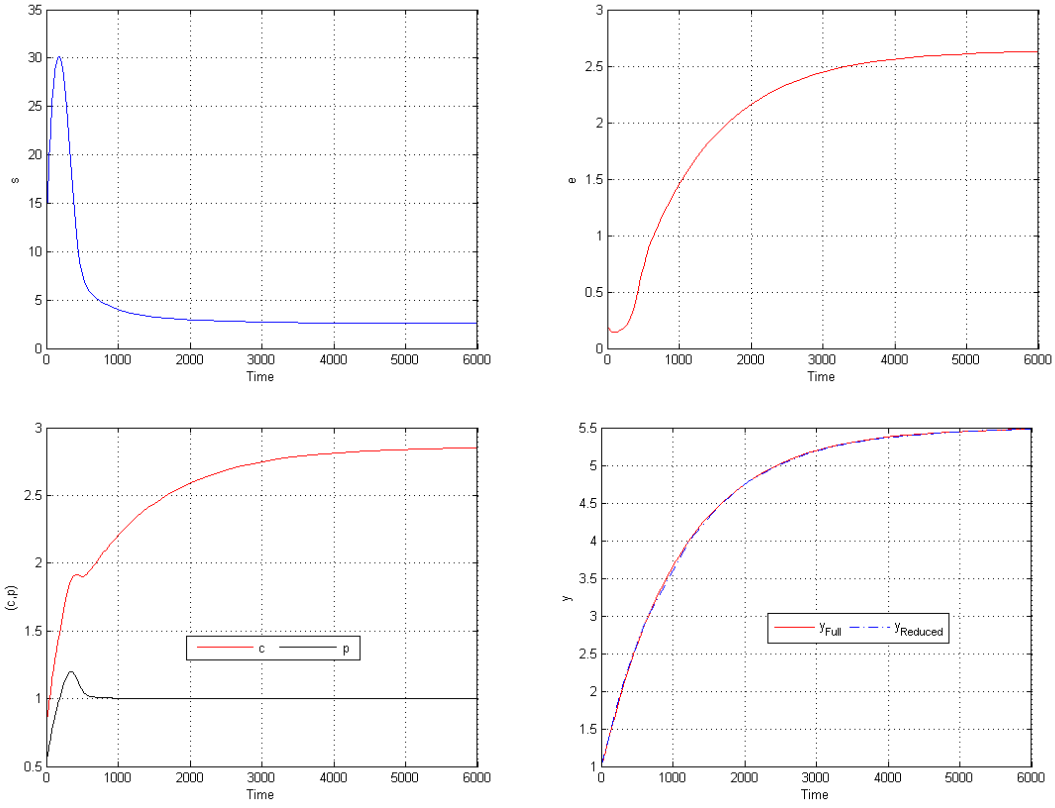

FIGURE 2.7.5: Taking these values of parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.36, k = 0.5, k_e = 0.005, \gamma_e = 0.0009, \theta_p = 0.4, s(t_0) = 12, e(t_0) = 0.2, c(t_0) = 0.8, p(t_0) = 1, y(t_0) = 1, u = 0.5$, in the beginning of the simulation we have $u > k_2 y(0)$, therefore first the equilibrium of the fast system does not exist; then the substrate s start increasing and because we have the production of the enzyme by the gene ($p > \theta_p$) it turns that $u < k_2 y(t)$ and finally the substrate s converges to the equilibrium.

2.7.3 Activation with the substrate of the enzyme

We suppose in the following that the substrate activates the production of the enzyme if it is above some threshold θ_s, see schema 2.7.7:

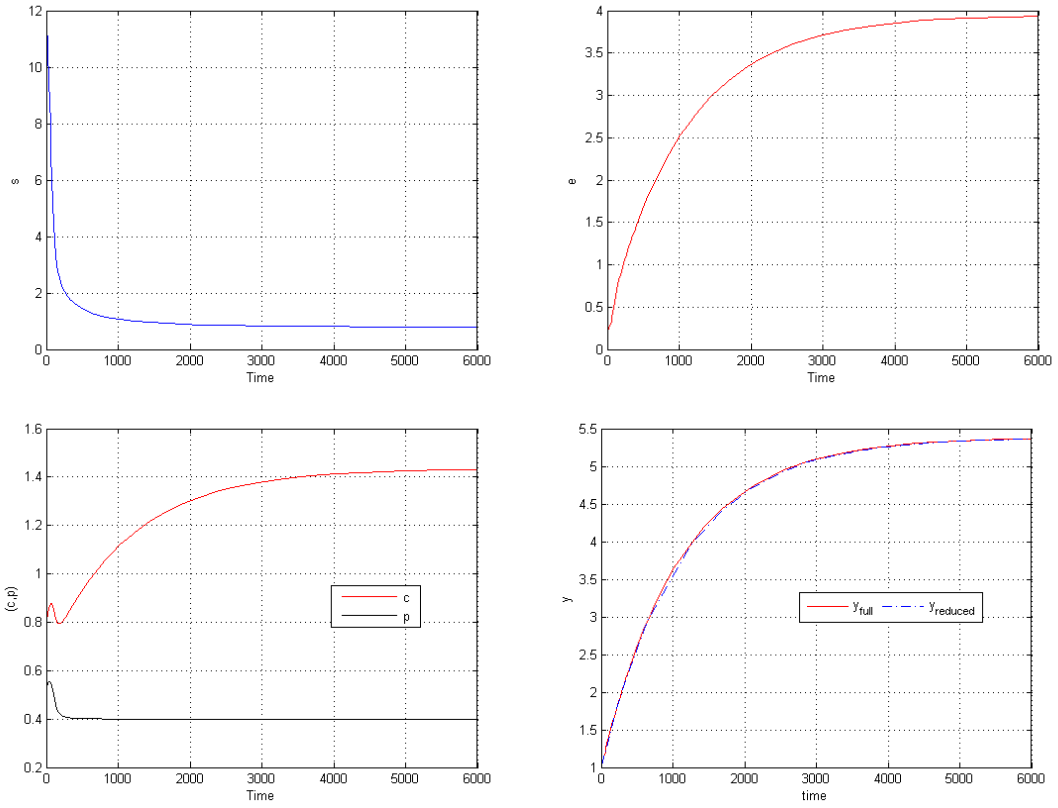

FIGURE 2.7.6: Taking these values of parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.36, k = 0.5, k_e = 0.005, \gamma_e = 0.0009, \theta_p = 0.2, s(t_0) = 12, e(t_0) = 0.2, c(t_0) = 0.8, p(t_0) = 1, y(t_0) = 1, u = 0.2$, in the beginning of the simulation we have $u < k_2 y(0)$, and y is increasing, therefore the equilibrium of the fast system exists and the behavior of the reduced system follows the full system.

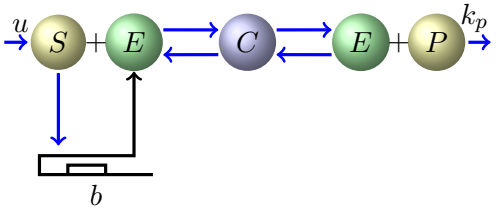

FIGURE 2.7.7: Coupled metabolic genetic system (activation of the production of enzymes by the substrate).

The dynamics of this system is given by:

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 se + k_{-1} c + \gamma_e c \\ \dfrac{de}{dt} = -k_1 se + k_{-1} c + k_2 c - k_{-2} pe + k_e \dfrac{s^n}{s^n + \theta_s^n} - \gamma_e e, \\ \dfrac{dc}{dt} = k_1 se - k_{-1} c - k_2 c + k_{-2} pe - \gamma_e c, \\ \dfrac{dp}{dt} = k_2 c - k_{-2} pe - kp \end{cases} \tag{2.7.10}$$

As previously, taking $y = e + c$, we transform system 2.7.10 to the following system with the form of Tikhonov:

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 s(y-c) + k_{-1}c + \gamma_e c \\ \dfrac{dc}{dt} = k_1 s(y-c) - k_{-1}c - k_2 c + k_{-2}p(y-c) - \gamma_e c \\ \dfrac{dp}{dt} = k_2 c - k_{-2}p(y-c) - kp \\ \dot{y} = k_e \frac{s^n}{s^n + \theta_s^n} - \gamma_e y \end{cases} \tag{2.7.11}$$

Using Tikhonov theorem we reduce the system 2.7.11 as follows:

$$\dot{y} = k_e \frac{s^n}{s^n + \theta_p^n} - \gamma_e y$$

The expression of s as a function of y are given by:

$$\begin{cases} c = \dfrac{(k + k_{-2}y)u}{k_2 k + k_{-2}u} \\ s = \dfrac{u + k_{-1}c}{k_1(y-c)} \\ \quad = \dfrac{(k_2 k + k_{-2}u + k_{-1}k)u + k_{-1}k_{-2}uy}{(k_2 k k_1 y - u k k_1)}. \end{cases} \tag{2.7.12}$$

we denote:

$$\begin{cases} \alpha = (k_2 k + k_{-2}u + k_{-1}k)u \\ \beta = k_{-1}k_{-2}u \\ \delta = k_2 k k_1 \\ \lambda = u k k_1 \end{cases} \tag{2.7.13}$$

Therefore, we can write:

$$s = \frac{\alpha + \beta y}{\delta y - \lambda} \tag{2.7.14}$$

Therefore, the dynamics of the reduced system is:

$$\dot{y} = k_e \frac{(\frac{\alpha+\beta y}{\delta y - \lambda})^n}{(\frac{\alpha+\beta y}{\delta y - \lambda})^n + \theta_s^n} - \gamma_e y.$$

The results of simulation are given in figure (2.7.8), and (2.7.9): in the two cases either $u > k_2 y(t)$ or $u < k_2 y(t)$, the evolution of the reduced system follows the full system.

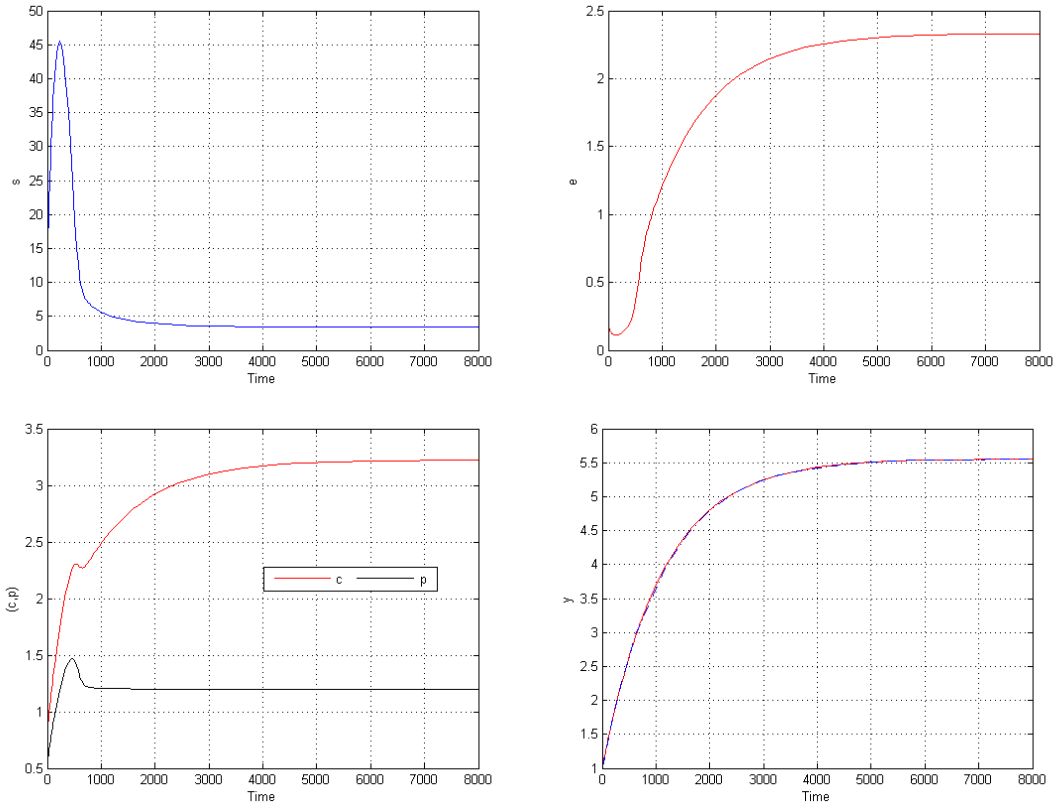

FIGURE 2.7.8: Taking these values of parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.36, k = 0.5, k_e = 0.005, \gamma_e = 0.0009, \theta_s = 0.2, s(t_0) = 12, e(t_0) = 0.2, c(t_0) = 0.8, p(t_0) = 1, y(t_0) = 1$, $u = 0.6$, first we have $u > k_2 y(0)$, where in the beginning the substrate s becomes high, after we have $s > \theta_s$ which leads to the synthesis of the enzyme by the gene and this leads also to $u < k_2 y(t)$, and therefore the full system converge toward an equilibrium.

2.7.4 A more general model with degradations (substrate is also degraded)

In the following we consider that all species are degraded (the substrate is also degraded with a rate k_s), and all the degradation of species and the input u are considered to be small with respect to all other parameters. We chose this model because the structure is very similar to the models of Chapter 4.

We consider for example the case that the final product activates the synthesis of the enzyme if it is above a threshold θ_p, therefore the dynamics of this system is:

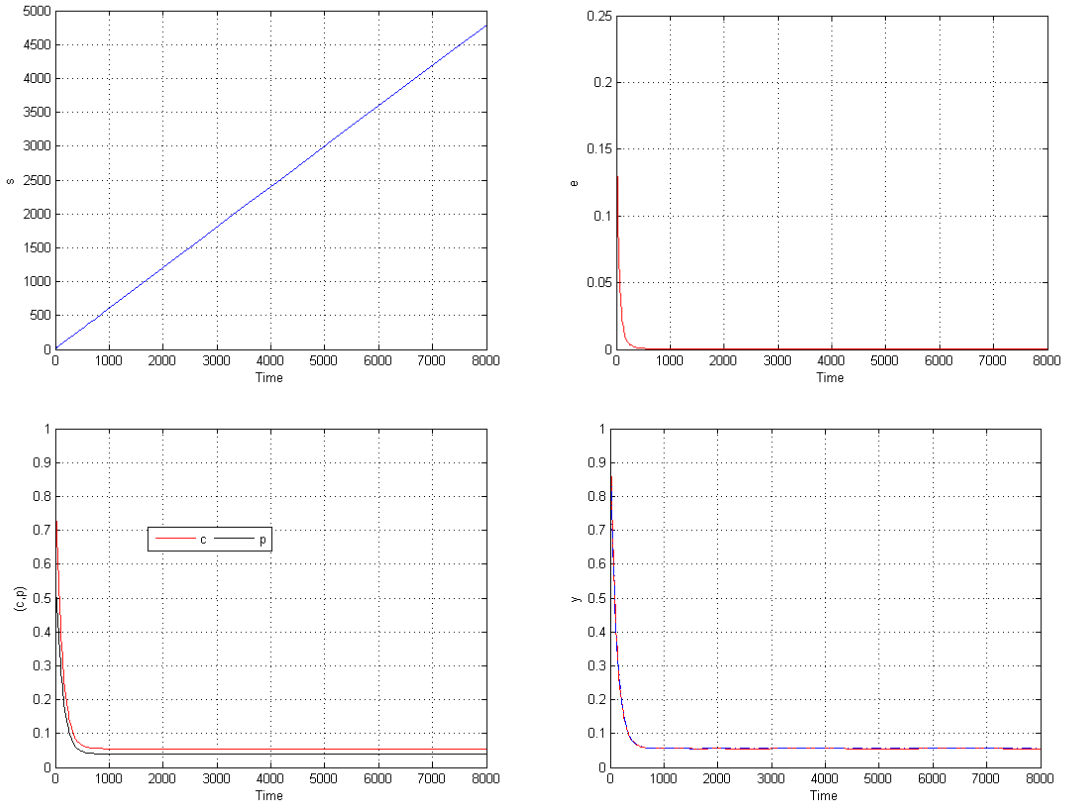

FIGURE 2.7.9: If we take these values of parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.36, k = 0.5, k_e = 0.0005, \gamma_e = 0.009, \theta_s = 0.4, s(t_0) = 12, e(t_0) = 0.2, c(t_0) = 0.8, p(t_0) = 1, y(t_0) = 1, u = 0.6$, we have a strong degradation of the enzyme, therefore the equilibrium disappears at infinity even if we have the synthesis of the enzyme when $s > \theta_s$.

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 se + k_{-1}c + \gamma_e c - k_s s \\ \dfrac{de}{dt} = -k_1 se + k_{-1}c + k_2 c - k_{-2}pe + k_e \dfrac{p^n}{p^n + \theta_p^n} - \gamma_e e + k_s c, \\ \dfrac{dc}{dt} = k_1 se - k_{-1}c - k_2 c + k_{-2}pe - \gamma_e c - k_s c, \\ \dfrac{dp}{dt} = k_2 c - k_{-2}pe - kp \end{cases} \tag{2.7.15}$$

To separate the full system into fast and slow subsystems, we consider:

$$\begin{cases} y = e + c \\ z = s + c + p \end{cases} \tag{2.7.16}$$

Introducing the first variable $y = e + c$ we get:

$$\begin{cases} \frac{ds}{dt} = -k_1 s(y-c) + k_{-1}c + u + \gamma_e c - k_s s \\ \frac{dc}{dt} = k_1 s(y-c) - k_{-1}c - k_2 c + k_{-2}p(y-c) - \gamma_e c - k_s c \\ \frac{dp}{dt} = k_2 c - k_{-2}p(y-c) - kp \\ \dot{y} = k_e \frac{p^n}{p^n + \theta_p^n} - \gamma_e y \end{cases} \tag{2.7.17}$$

Introducing also the variable $z = s + c + p$ we obtain:

$$\begin{cases} \frac{ds}{dt} = -k_1 s(y-c) + k_{-1}c + u + \gamma_e c - k_s s \\ \frac{dc}{dt} = k_1 s(y-c) - k_{-1}c - k_2 c + k_{-2}p(y-c) - \gamma_e c - k_s c \\ \frac{dp}{dt} = k_2 c - k_{-2}p(y-c) - kp \\ \frac{dz}{dt} = u - kp - k_s s - k_s c \\ \frac{dy}{dt} = k_e \frac{p^n}{p^n + \theta_p^n} - \gamma_e y \end{cases} \tag{2.7.18}$$

where the fast part is given by this system:

$$\begin{cases} \frac{ds}{dt} = -k_1 s(y-c) + k_{-1}c \\ \frac{dc}{dt} = k_1 s(y-c) - k_{-1}c - k_2 c + k_{-2}p(y-c) \\ \frac{dp}{dt} = k_2 c - k_{-2}p(y-c) \end{cases} \tag{2.7.19}$$

Notice that the dynamics of the evolution of the fast part (given by variables (s, c, p)) is exactly represented by the closed system of Michaelis-Menten, and remind that this system is globally stable towards the equilibrium point which depends on the hyperplane H_0. In our case the quasi-steady state equilibrium varies slowly due to the variation of z or y, on the hyperplane $H_{(z,y)} = \left\{(s, c, p) \in \Re^3 : \{s + c + p = z(t)\}\right\}$.

The slow subsystem is given by:

$$\begin{cases} \frac{dz}{dt} = u - kp - k_s(s + c) \\ \frac{dy}{dt} = k_e \frac{p^n}{p^n + \theta_p^n} - \gamma_e y \end{cases} \tag{2.7.20}$$

Therefore, we have to express p and s as a function of z and y.

For the fast part we obtain at equilibrium:

$$\begin{cases} s = \dfrac{k_{-1}c}{k_1(y-c)} \\ p = \dfrac{k_2 c}{k_{-2}(y-c)} \end{cases} \tag{2.7.21}$$

We have also:

$$\begin{cases} z &= s + c + p \\ &= \frac{k_{-1}c}{k_1(y-c)} + c + \frac{k_2 c}{k_{-2}(y-c)} \end{cases} \tag{2.7.22}$$

Therefore, if we keep this equation 2.7.22 as an algebraic equation giving c as a function of z (it is easy to check that it has a unique positive solution, see the study of the closed Michaelis Menten equation), we get the following reduced system:

$$\begin{cases} \dot{z} = u - k\frac{k_2 c}{k_{-2}(y-c)} - k_s(\frac{k_{-1}c}{k_1(y-c)} + c) \\ \dot{y} = k_e \frac{(\frac{k_2 c}{k_{-2}(y-c)})^n}{(\frac{k_2 c}{k_{-2}(y-c)})^n + \theta_p^n} - \gamma_e y \\ z = \frac{k_{-1}c}{k_1(y-c)} + c + \frac{k_2 c}{k_{-2}(y-c)} \end{cases} \tag{2.7.23}$$

The results of simulations of the full system and the reduced system are given in figure 2.7.10 where we can show that the reduced system follows the full system and the quasi-steady state approximation is valid.

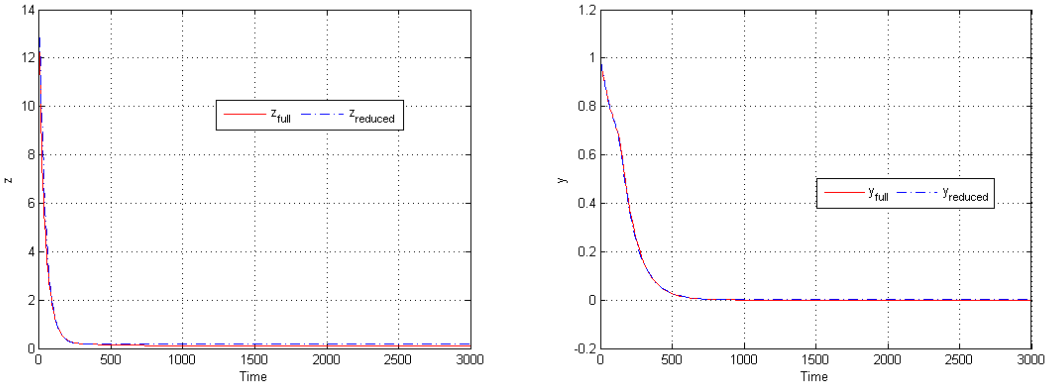

FIGURE 2.7.10: If we take this values of parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.36$,u=0.004, $k_s = 0.04, k = 0.005, \gamma_e = 0.009, k_e = 0.005, \theta_p = 0.4, s(t_0) = 12, e(t_0) = 0.2, c(t_0) = 0.8, p(t_0) = 1, y(t_0) = 1$, $n = 5$, the behavior of the reduced system follows the behavior of the full systems.

2.8 Conclusion

We applied tools from monotone systems theory and compartmental systems theory to the study of stability of the full open and closed Michaelis-Menten system, and of metabolic chains. We were able to show stability in a simple and global way. For the open systems, we showed that if the input u is too large, there is no equilibrium. We think that these kind of tools could be applied to other metabolic systems. A simple generalization to an open chain of full reversible Michaelis-Menten systems is investigated here. Different examples of coupled metabolic-genetic are given and reduced using Tikhonov theorem. Finally we conclude that the reduced system or the approximations using Tikhonov theorem maybe becomes not valid if the equilibrium of the fast system does not exist (disappears at infinity).

Chapter 3

Transcription translation coupled models for gene expression with inputs.

The aim of this chapter is to present and to analyze coupled transcription-translation models developed in ([42]), adding some inputs and outputs, which are a detailed representation of the process describing how the information in DNA turns into proteins in a cell. Our goal is to study the global dynamical behaviour and stability using monotone systems theory.

The biological and modeling aspects of this work are done in collaboration with D. Ropers and E. Grac (IBIS INRIA Grenoble).

3.1 Introduction

The genetic information of a cell is encoded in double-stranded DNA (acronym for *deoxyribonucleic acid*) molecules (see [1]). The key paradigm of molecular biology is called the *central dogma of molecular biology*: "DNA makes RNA, RNA makes protein, and proteins make the cell". *Proteins* are the primary components of living things. Among other roles, they form receptors that endow the cell with sensing capabilities, actuators that make muscles move, detectors for the immune response, enzymes that catalyze chemical reactions, and switches that turn genes on or off.

The term *gene expression* refers to the process by which genetic information its transformed into working proteins. The main steps are transcription from DNA to RNA and

translation from RNA into functional proteins, but several intermediate steps usually take place as well. All these steps are also highly regulated.

The transcription is a copy of the information of the gene on the DNA strand into mRNA (messenger RNA), where the polymerase has a key role. The second phase of the process is the translation from RNA to linear amino acid sequences, and folding of these amino acids into functional proteins, via the ribosomal units, see [1].

Usually, classical models of gene expression only involve concentrations of mRNAs and of proteins. The RNA polymerase and ribosomes, for example, are always supposed to be in sufficient quantity, and therefore non limiting. Yet, some works emphasize the important role of the global machinery for gene expression (see [8] for an example). It is therefore interesting to build detailed models involving the main actors of the transcription-translation process, such as RNA polymerase and ribosomes. Some partial detailed models of this kind have been developed, see [42] for an example.

In the following, in a first step we investigate the dynamical behaviour of the model of gene transcription and the model of translation developed in [42], with taking some inputs and outputs. Then we focused on the coupled transcription-translation model, where we combine these two models. The coupled transcription-translation model is a part of gene expression machinery ([8],[52]). This coupled model is not regulated, and is meant to be included in a bigger model. Our goal is to study the global dynamical behaviour with the help of simple but powerful tools (monotone systems theory).

3.2 The gene transcription model

We consider in the following the first step called transcription, where RNA polymerase binds on a specific site of the DNA strand, and moves along the strand, producing messenger RNA. In a first part in this section we study a “closed” model, closed meaning that some quantities (the total polymerase) is conserved. Then we add input and output to the model, and study its behaviour using monotone systems theory.

3.2.1 The ”closed” transcription model

In the following the reaction scheme for the model of transcription presented in [42] is shown, and we write the system of ODEs associated to the model. Details of the models are given in [42].

3.2.2 Description of the model

A single gene with length l is considered. RNA polymerase P with σ factor binds to the specific DNA binding site D. After binding, the polymerase clears the promoter (parameter k_c) and moves along the DNA (parameter k_t). Complexes Y and Y^i describe the moving polymerase which binds to nucleotides along the strand. The completed RNA molecule is subject to degradation (parameter k_m). Nucleotides are supposed to be in excess, and their concentrations are included in the parameters. All variables are described by their concentrations. The scheme given in [42] is:

$$\begin{aligned}
\sigma + P + D &\underset{k_-}{\overset{k_+}{\leftrightarrows}} PD \\
PD &\xrightarrow{k_c} Y + D + \sigma \\
Y + Nu &\xrightarrow{k_t} Y^1 \\
Y^1 + Nu &\xrightarrow{k_t} Y^2 \\
&\vdots \\
Y^{l-1} + Nu &\xrightarrow{k_t} P + RNA \\
RNA &\xrightarrow{k_r} \phi
\end{aligned}$$

We can derive the following system from the reaction scheme (following classical mass-actions kinetics):

$$\begin{aligned}
\dot{c} &= k_+ \, p \, d - k_- \, c - k_c \, c \\
\dot{d} &= -k_+ \, p \, d + k_- \, c + k_c \, c \\
\dot{p} &= -k_+ \, p \, d + k_t \, y^{l-1} + k_- \, c \\
\dot{y} &= k_c \, c - k_t \, y \\
\dot{y}^1 &= k_t \, y - k_t \, y^1 \\
&\vdots \\
\dot{y}^{l-1} &= k_t \, y^{l-2} - k_t \, y^{l-1} \\
\dot{m} &= k_t \, y^{l-1} - k_m \, m
\end{aligned}$$

where p, d, c, y , y^i and m are the concentrations of P, D, PD, Y, Y^i and RNA respectively.

Note that the system has to fulfill two mass conservations (because it is closed), describing the total concentration of promoter site d_0 and the total concentration of polymerase M_0.

$$\begin{aligned} \dot{c} + \dot{d} &= 0 \\ \dot{c} + \dot{p} + \dot{y} + \dot{y}^1 + \ldots + \dot{y}^{l-1} &= 0 \end{aligned} \tag{3.2.1}$$

which leads to

$$\begin{aligned} d + c &= d_0 \\ c + p + y + y^1 + \ldots + y^{l-1} &= M_0 \end{aligned} \tag{3.2.2}$$

It is important to notice that the order of the system depends on the parameter l (the length of the gene being transcribed) and that the system is nonlinear. The equations for complexes y^i represent a linear subsystem which could be interpreted as a delay. The two mass conservations (3.2.2) play an important role in the dynamics of the whole system and should be treated with care. We also remark that the ratio l/k_t is constant, because it has a biological interpretation (see [42]) of the time taken by the polymerase from the start to the end of the gene.

It is easily checked that the variable stay positive if the initial conditions are positive (the positive orthant is forward invariant), and that all variables are bounded (because of the mass conservation in particular, and of the positivity of variables) by bounds depending on the initial conditions.

3.2.3 Equilibrium

The aim of this section is to compute the steady state of the system. Taking into account (3.2.2) we can reduce the original system to

$$\begin{aligned} \dot{c} &= k_+ \, p \, (d_0 - c) - k_- \, c - k_c \, c \\ \dot{p} &= -k_+ \, p \, (d_0 - c) + k_t \, y^{l-1} + k_- \, c \\ \dot{y} &= k_c \, c - k_t \, y \\ \dot{y}^1 &= k_t \, y - k_t \, y^1 \\ \dot{y}^2 &= k_t \, y^1 - k_t \, y^2 \\ &\vdots \\ \dot{y}^{l-1} &= k_t \, y^{l-2} - k_t \, y^{l-1} \\ \dot{m} &= k_t \, y^{l-1} - k_m \, m \end{aligned} \tag{3.2.3}$$

We get the following equations for the steady state:

$$\begin{aligned} y^{l-1} = \ldots = y^1 = y &= \frac{k_c}{k_t}\, c \\ c &= \frac{k_+\, p\, d_0}{k_+\, p + k_- + k_c} \\ m &= \frac{k_t}{k_m} y^{l-1} \end{aligned} \tag{3.2.4}$$

We rewrite

$$\begin{aligned} c &= d_0\, \frac{p}{p + K_1} \\ K_1 &= \frac{k_- + k_c}{k_+} \end{aligned} \tag{3.2.5}$$

Replacing (3.2.5) and (3.2.4) in (3.2.2) we obtain

$$l\, \frac{k_c}{k_t}\, d_0\, \frac{p}{p + K_1} + p + d_0\, \frac{p}{p + K_1} = M_0 \tag{3.2.6}$$

We remark that the left side of (3.2.6) is an increasing function of p, which is zero for p equals to zero, and tends to infinity when p tends to infinity; therefore we can deduce that (3.2.6) has a unique solution which depends on M_0 and d_0. We define the hyperplane $H_0 = \{(c, p, y, y^1, \ldots, y^{l-1}) \in \Re^{l+2} : \{c + p + y + y^1 + \ldots$
$+ y^{l-1} = M_0 > 0\}$.

Therefore system has a unique steady state for each hyperplane H_0. The whole steady state can be obtained after solving (3.2.6) for p. In the following sections we are going to study the stability of this equilibrium on the invariant hyperplane.

3.2.4 Global stability of the equilibrium of the closed transcription model

In the following we study the global stability of the equilibrium. Last variable m has no influence on the other variables of the system, and moreover

$$\dot{m} = k_t\, y^{l-1} - k_m\, m \tag{3.2.7}$$

Therefore it is easy to show with classical arguments that, if the system with $(c, p, y, \ldots, y^{l-1})$ is globally asymptotically stable (w.r.t. the invariant hyperplane), then the full system (with m) will be such.

Taking the system (3.2.3) without r, This system is closed, in the sense that $\dot{c}+\dot{p}+\dot{y}+\dot{y}^1+.....+\dot{y}^{L-1}=0$. The Jacobian matrix $J(c,p,y,y^1,\ldots,y^{L-1})$

$$= \begin{pmatrix} -(k_- + k_c - k_+ p) & k_+ (d_0 - c) & 0 & \ldots & 0 \\ k_- + k_+ p & -k_+ (d_0 - c) & 0 & \ldots & k_t \\ k_c & 0 & -k_t & \ldots & 0 \\ 0 & 0 & k_t & \ldots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \ldots & -k_t \end{pmatrix} \tag{3.2.8}$$

is a compartmental matrix which means that :

$$\begin{cases} J_{ii}(c,p,y,y^1,\ldots,y^{L-1}) \leq 0 & for \ \ all \ \ i, \\ J_{ij}(c,p,y,y^1,\ldots,y^{L-1}) \geq 0 & for \ \ all \ \ i \neq j, \\ -J_{jj}(c,p,y,y^1,\ldots,y^{L-1}) \geq \sum_{i \neq j} J_{ij}(c,p,y,y^1,\ldots,y^{L-1}) & for \ \ all \ \ j \end{cases}$$

We can also easily to check that the graph of the Jacobian matrix is strongly connected. The interaction graph associated with the Jacobian matrix (3.2.8) is shown in Figure (3.2.1)

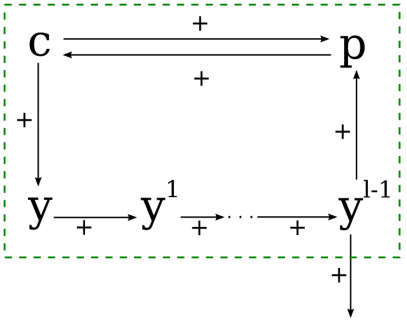

FIGURE 3.2.1: Interaction Graph of system (3.2.3).

Therefore, we can apply Property 5 [3] to obtain this theorem:

Theorem 3.1. *Let* $z(c,p,y,y^1,\ldots,y^{L-1}) = c+p+y+y^1+...+y^{L-1}$ *the (fixed) total concentration of the closed system.*
The Jacobian matrix of the system is compartmental and irreducible (its graph is strongly connected); then for any $z > 0$, *hyperplane* $H_z = \{(c,p,y,y^1,\ldots,y^{L-1}) \in \Re_+^{L+2} : z(c,p,y,y^1,\ldots,y^{L-1}) = z > 0\}$ *is forward invariant and contains a unique equilibrium, globally stable in* H_z.

The proof of this theorem is with a Lyapunov function (for more details of this proof see [39]). We conclude that the equilibrium is unique on the hyperplane and globally asymptotic stable with respect to this hyperplane H_z, moreover it is also locally asymptotic stable with respect to this hyperplane H_z.

In figure (3.2.2) we show the evolution of variables c, p, and y along the space state for two different initial conditions which lie in the same hyperplane H_z. Both trajectories are going toward the same equilibrium with damped oscillations. For illustration purposes we have simulated (3.2.3) for example with these parameters: $k_+ = 1000$, $k_- = 700$, $k_c = 1.5$, $k_t = 4.91$, $L = 20$, $d_0 = 7.96$.

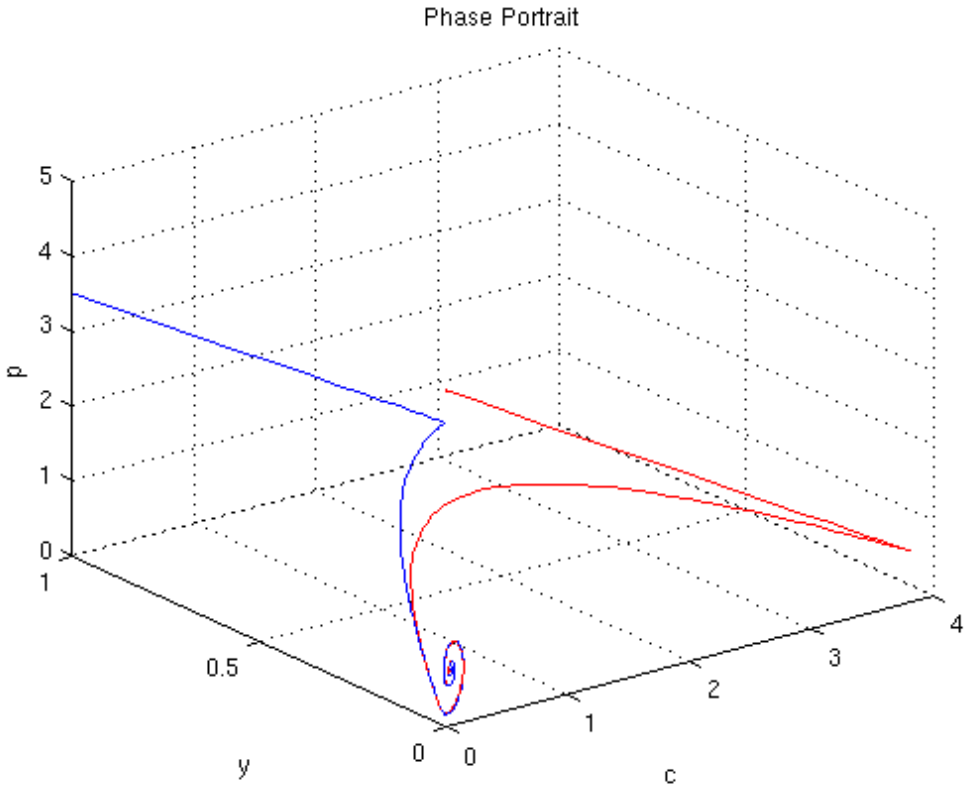

FIGURE 3.2.2: Simulation of the system (3.2.3), with two different initial conditions lying in the same hyperplane.

3.2.5 The "open" transcription model

Until now we have studied a model where the total polymerase remained constant. This is not very realistic: in fact the polymerase is made by the cellular global machinery, and also degraded [1].

3.2.6 Adding Synthesis and Degradation to the free polymerase

In this section we are going to suppose that the free polymerase has a synthesis input term k and a degradation term k_p; these terms are meant to represent the input and output for polymerase, coming from other subsystems of the cell; we will study the stability of this "open" model.

3.2.7 Equations of the model

The new system, considering the synthesis and degradation terms for variable p is

$$\begin{aligned}
\dot{c} &= k_+\, p\,(d_0 - c) - k_-\, c - k_c\, c \\
\dot{p} &= -k_+\, p\,(d_0 - c) + k_t\, y^{l-1} + k_-\, c + \mathbf{k} - \mathbf{k_p}\,\mathbf{p} \\
\dot{y} &= k_c\, c - k_t\, y \\
\dot{y}^1 &= k_t\, y - k_t\, y^1 \\
\dot{y}^2 &= k_t\, y^1 - k_t\, y^2 \\
&\vdots \\
\dot{y}^{l-1} &= k_t\, y^{l-2} - k_t\, y^{l-1} \\
\dot{m} &= k_t\, y^{l-1} - k_m\, m
\end{aligned} \tag{3.2.9}$$

We will introduce a new variable z which has no real physiological justification but represents the total polymerase which was constant in the closed system and is now varying.

$$z = c + p + y + y^1 + \ldots + y^{l-1}$$

the dynamics of z is

$$\dot{z} = k - k_p\, p$$

3.2.8 Equilibrium

At steady state, we have:

$$\begin{aligned} y^{l-1} = \ldots = y^1 = y &= \frac{k_c}{k_t}\, c \\ c &= d_0\, \frac{p}{p + K_1} \\ p &= \frac{k}{k_p} \\ m &= \frac{k_t}{k_m} y^{l-1} \end{aligned} \tag{3.2.10}$$

Therefore there is a single steady state for the full system.

3.2.9 Global stability of the equilibrium of the open transcription model

In a first step, we study the system without m, for the same reasons as above.

The system 3.2.9 is monotone; thus we can apply the proposition A.1 of the appendix to study the stability of the equilibrium, i.e. fulfill conditions meaning that if there is a point in the space state greater than the equilibrium point, (with respect to the usual partial ordering) where all the derivatives are non-positive, then the trajectory issued from this point is always decreasing, and converges toward the equilibrium. Similarly, if there is a point smaller than the equilibrium where the derivatives are non-negative, then the solution from this point increases until the equilibrium. The whole hyper-rectangle built with these two points is invariant, and all trajectories initiating in this rectangle converge towards the unique equilibrium.

For the lower vertex of the hyper-rectangle we will choose $c = y = y^1 = \cdots = y^{l-1} = p = 0$ and obtain the velocities

$$\dot{c} = \dot{y} = \dot{y}^1 = \cdots = \dot{y}^{l-1} = 0, \quad \dot{p} = k \tag{3.2.11}$$

which is positive.

For the upper vertex, we want to find a point as large as possible, such that the vector field is nonpositive. c is lower than d_0 (because of the first integral (4.3.9)) so we are going to take $c = d_0$ which leads to

$$\dot{c} = -(k_- + k_c)\, d_0 \tag{3.2.12}$$

which is negative.

We also need to fulfill

$$\begin{aligned} y &\geq \frac{k_c}{k_t}\, d_0 \\ y^1 &\geq y \\ &\vdots \\ y^{l-1} &\geq y^{l-2} \\ k_p p &\geq k_t\, y^{l-1} + k_-\, d_0 + k \end{aligned} \tag{3.2.13}$$

then we can reduce the set of inequalities (3.2.13) to

$$\begin{aligned} y^{l-1} = \cdots = y^1 = y \geq \frac{k_c}{k_t}\, d_0 \\ k_p p \geq k_t\, y^{l-1} + k_-\, d_0 + k \end{aligned}$$

These two inequalities are easy to fulfill.

Finally we can choose the upper vertex such that $c = d_0$ and the other variables may be as large as wanted, and fulfilling the above inequalities. It is also easy to check that the system is locally asymptotically stable on the hyperplane (the linearized system at the equilibrium is a compartmental matrix). We can conclude that the equilibrium point of the full system is globally asymptotically stable in all the admissible space.

Many models of gene networks exist ([2, 17, 70]), but very few of them include the basic elements of cell machinery, such as polymerase. For an application of monotone systems theory for chemical chains, see [19]. We believe that our tools of monotonicity are well adapted to biological problems, as noticed by other studies [60]. In spite of the nonlinear form and number of equations, we are able to study the system in a simple and global way.

3.3 The closed translation model

Now we describe the translation system, using ribosomes to make proteins from mRNA.

The process of translation could be initiated from every nascent mRNA as it is shown in ([42]); to simplify, in the following we suppose that the proteins are synthesized from completed mRNA only, with length h; the reaction scheme is the following:

$$
\begin{aligned}
R \;+\; RNA' &\overset{k'_+}{\underset{k'_-}{\leftrightarrows}} RRNA \\
RRNA &\overset{k_w}{\to} X \;+\; RNA' \\
X \;+\; tRNA^* &\overset{k'_t}{\to} tRNA \;+\; X^1 \\
X^1 \;+\; tRNA^* &\overset{k'_t}{\to} tRNA \;+\; X^2 \\
&\vdots \\
X^{h-1} \;+\; tRNA^* &\overset{k'_t}{\to} R \;+\; S \\
S &\overset{k_s}{\to} \phi
\end{aligned}
\tag{3.3.1}
$$

where RNA$'$ represents a molecule of $mRNA$ with length h and with a free ribosome binding site. RRNA represents the ribosome bound to its binding site. X and X^j describe the moving ribosome on the completed RNA. R is the free ribosome. S is the protein which is being translated.

The system of ODEs associated to the model is:

$$
\begin{aligned}
\dot{w} &= k'_+ r\, m - k'_- w - k_w w \\
\dot{m} &= -k'_+ r\, m + k'_- w + k_w w \\
\dot{r} &= -k'_+ r\, m + k'_- w + k'_t x^{h-1} \\
\dot{x} &= k_w w - k'_t x \\
\dot{x}^1 &= k'_t x - k'_t x^1 \\
&\vdots \\
\dot{x}_{h-1} &= k'_t x^{h-2} - k'_t x^{h-1} \\
\dot{s} &= k'_t x^{h-1} - k_s s
\end{aligned}
\tag{3.3.2}
$$

where w, m, r , s, x and x^i are the concentration of RRNA, RNA$'$, R, S, X and X^i respectively. Notice as previously that we have the following equations for the conservation of total amount of mRNA (q_0) and ribosomes (R_0)

$$
\begin{aligned}
w + m &= q_0 \\
r + w + x + x^1 + \cdots + x^{h-1} &= R_0
\end{aligned}
\tag{3.3.3}
$$

the reduced system becomes:

$$\begin{aligned}
\dot{w} &= k_{+}^{'}\, r\,(q_0 - w) - k_{-}^{'}\, w - k_w\, w \\
\dot{r} &= -k_{+}^{'}\, r\,(q_0 - w) + k_{-}^{'}\, w + k_t^{'}\, x^{h-1} \\
\dot{x} &= k_w\, w - k_t^{'}\, x \\
\dot{x}^1 &= k_t^{'}\, x - k_t^{'}\, x^1 \\
&\vdots \\
\dot{x}_{h-1} &= k_t^{'}\, x^{h-2} - k_t^{'}\, x^{h-1} \\
\dot{s} &= k_t^{'}\, x^{h-1} - k_s\, s
\end{aligned} \tag{3.3.4}$$

The translation system is quite similar to the system of the transcription system above. The study of the equilibrium and the stability is exactly the same as before, therefore will not be given here. We define the hyperplane $G = \left\{(w, r, x, x^1, \ldots x^{h-1}) \in \Re^{h+2} : w + r + x + x^1 + \ldots + x^{h-1} = R_0\right\}$. The final result is that this system (4.3.9) has a unique equilibrium on this invariant hyperplane, which is globally asymptotically stable.

3.4 A coupled transcription-translation model

The aim of this section is to analyze a transcription-translation model, which couples the two models above.

3.4.1 Equations of the model

We can couple both transcription and translation processes, to obtain the following full system (3.4.1).

$$
\begin{aligned}
\dot{c} &= k_+\, p\,(d_0 - c) - k_-\, c - k_c\, c \\
\dot{p} &= -k_+\, p\,(d_0 - c) + k_t\, y^{l-1} + k_-\, c + k - k_p\, p \\
\dot{y} &= k_c\, c - k_t\, y \\
\dot{y}^1 &= k_t\, y - k_t\, y^1 \\
\dot{y}^2 &= k_t\, y^1 - k_t\, y^2 \\
&\vdots \\
\dot{y}^{l-1} &= k_t\, y^{l-2} - k_t\, y^{l-1} \\
\dot{w} &= k_+^{'}\, r\, m - k_-^{'}\, w - k_w\, w \\
\dot{m} &= -k_+^{'}\, r\, m + k_-^{'}\, w + k_w\, w + k_t\, y^{l-1} - k_m\, m \\
\dot{r} &= -k_+^{'}\, r\, m + k_-^{'}\, w + k_t^{'}\, x^{h-1} \\
\dot{x} &= k_w\, w - k_t^{'}\, x \\
\dot{x}^1 &= k_t^{'}\, x - k_t^{'}\, x^1 \\
&\vdots \\
\dot{x}^{h-1} &= k_t^{'}\, x^{h-2} - k_t^{'}\, x^{h-1} \\
\dot{s} &= k_t^{'}\, x^{h-1} - k_s\, s
\end{aligned}
\tag{3.4.1}
$$

The model is hierarchically built: the first transcription model is an input to the second translation model, that can be seen by the term $k_t\, y^{l-1}$ in $\dot{m}$.

3.4.2 Stability of the coupled model

We briefly describe the stability properties of the whole model. We can use the global stability property of the first transcription model, and the globally stable equilibrium of this model is used as an input for the second step of the whole model. This kind of argument can be rigorously justified from a mathematical point of view with theorems concerning the stability of hierarchical systems (see [68]). In particular, it is valid when all the variables are bounded, which is the case here, as it can be easily checked.

Now if we use the equilibrium y^{l-1^*} to input it in the second model, and introduce the variable $q = m + w$, we obtain

$$
\dot{q} = \dot{m} + \dot{w} = k_t\, y^{l-1^*} - k_m\, m \tag{3.4.2}
$$

Replacing variable m by $(q - w)$, and introducing also the variable z the system (3.4.1) becomes:

$$
\begin{aligned}
\dot{c} &= k_+\, p\,(d_0 - c) - k_-\, c - k_c\, c \\
\dot{p} &= -k_+\, p\,(d_0 - c) + k_t\, y^{l-1} + k_-\, c + k - k_p\, p \\
\dot{y} &= k_c\, c - k_t\, y \\
\dot{y}^1 &= k_t\, y - k_t\, y^1 \\
\dot{y}^2 &= k_t\, y^1 - k_t\, y^2 \\
&\vdots \\
\dot{y}^{l-1} &= k_t\, y^{l-2} - k_t\, y^{l-1} \\
\dot{z} &= k - k_p\, p \\
\dot{w} &= k_+^{'}\, r\,(q - w) - k_-^{'}\, w - k_w\, w \\
\dot{r} &= -k_+^{'}\, r\,(q - w) + k_-^{'}\, w + k_t^{'}\, x^{h-1} \\
\dot{x} &= k_w\, w - k_t^{'}\, x \\
\dot{x}^1 &= k_t^{'}\, x - k_t^{'}\, x^1 \\
&\vdots \\
\dot{x}^{h-1} &= k_t^{'}\, x^{h-2} - k_t^{'}\, x^{h-1} \\
\dot{q} &= k_t\, y^{l-1} - k_m\,(q - w) \\
\dot{s} &= k_t^{'}\, x^{h-1} - k_s\, s
\end{aligned}
\tag{3.4.3}
$$

Unfortunately, this model is not monotone anymore, as the translation model alone (with q constant) is, and we were not able to obtain global stability results for this model as we did above for the open transcription model using the monotone systems theory.

3.5 Conclusion

The transcription or the translation model is of large dimensions and can be difficult to handle. Fortunately, it is monotone: using monotone systems theory, it was possible to investigate the global stability of each model separately. The coupled transcription-translation is not monotone anymore; simplifications are necessary, the reduced system of this model is given in [4]. To prevent repetitions, the approach of reduction is not given here but it will be our main interest in the next chapter (for a similar model): we will try to reduce this system (with adding a loop for the polymerase) into simpler monotone system using Tikhonov theorem and finally we will be able to analyze and to study it.

Chapter 4

Reduction and stability analysis of a transcription-translation model of RNA polymerase

The aim in this chapter is to analyze the dynamical behaviour of models of gene transcription-translation for the synthesis of RNA polymerase in a cell, with a closed loop from the produced RNA polymerase (end-product) to the transcription step (RNA polymerase is needed to transcribe its own gene). Using monotone system theory we study a reduced version of this model with two variables (mRNA and protein), and show that it has either a single globally stable trivial equilibrium in $(0, 0)$, or has an unstable zero equilibrium and a globally stable positive one. This dynamical behaviour can be related to biological observations on the bacterium *Escherichia coli*. The same result holds for a class of qualitatively defined functions. We use some mathematical tools from monotone systems with a decreasing Jacobian matrix to obtain some generalizations of this result, specially for a more general model with n dimensions.

The biological and modeling aspects of this work are done in collaboration with D. Ropers and E. Grac (IBIS INRIA Grenoble).

4.1 Introduction

The central dogma of molecular biology is that "DNA makes RNA and RNA makes proteins", which are the primary components of cells, see [1]. Transcription is the first step of gene expression, in which a fragment of DNA (the gene) is copied into a messenger RNA (mRNA) by the RNA polymerase. The mRNA is translated into

proteins by ribosomes in the second step. In prokaryotic cells like bacteria, transcription and translation take place in the same compartment. As a consequence, ribosomes can translate nascent mRNAs being elongated by the RNA polymerase.

In this chapter, we focus on a coupled transcription-translation model for the expression of RNA polymerase of *Escherichia coli*, which is a small part of the gene expression machinery ([8]). There is only one type of RNA polymerase in this bacterium, on which the transcription of its own genes and all cellular genes depends. The coupled model being too difficult to handle because of its high dimension, we reduce it into a much simpler system and study the mathematical properties of the reduced model. To investigate the stability of the reduced system, we use monotone system theory. Monotone system means that the dynamical system is such that the partial order in dimension n between two solutions is conserved (see [58]). This kind of qualitative tools for proving stability are well adapted to the study of biological models ([60]). Monotone systems have strong properties of convergence towards equilibria, and cannot (for example) exhibit stable periodic oscillations. Moreover, they are characterized by the fact that the Jacobian matrix of the system is nonnegative outside the main diagonal: it means basically that every variable has a positive influence on the velocity of the others, and this qualitative property is often met in some classes of biological systems. The second tool is more particular, and linked to the concavity of the functions used in the differential equation [55]: the Jacobian matrix has to be decreasing with respect to the variables (in dimension n). These tools are particularly appealing, in our opinion, because they are qualitative (they do not depend too much of the values of parameters), and give very strong results concerning the global dynamical behaviour of the systems ([38]).

These tools are known, have been already applied for biological systems; population dynamics [56], chemical networks ([19])... The authors have worked with monotone systems theory on metabolic-genetic networks ([5]) and on detailed models for gene expression, without any loop ([4, 6]). Yet, in our knowledge, this theorem on concave and monotone systems has not been used in the context of detailed gene expression models, where we obtain that some functions are given by rather complex expressions resulting from mass-balance for substrates.

We present the full model and its reduction in the next section. We analysis of the different time scales in the evolution of the variables of the full system using the parameters built from the literature and from the biological experiments. We can write the full system into two different Fast and Slow subsystems. Applying Tikhonov's Theorem we put the fast subsystem to its quasi steady state and we succeed to reduce the full system, we obtain a reduced model having a similar dynamical dynamical behavior. Then

we analyze the reduced system, checking the concavity and monotonicity assumptions for this model. Using the concavity and monotonicity theory, we show that the trivial equilibrium is either globally stable (in that case no other equilibrium exits) or locally unstable, and that implies the existence and uniqueness of a positive equilibrium, which is globally stable with respect to the positive orthant. it is interesting to remark that the resulting Jacobian matrix is still cooperative and decreasing for such models, even with qualitative hypotheses. Finally a generalization of this results for a more general model with n dimensions and some biological results are given.

4.2 The coupled transcription-translation model of RNA polymerase

4.2.1 Description of the model

The reaction scheme of the transcription-translation model for the synthesis of RNA polymerase in a cell is given by Figure (4.2.1). For simplification reasons, we consider the RNA polymerase to be encoded from a single gene.

This model is inspired from the models given in [42]. During transcription, the RNA polymerase P with the transcription-initiation factor σ recognizes and binds to its specific DNA binding site D in the promoter region. After binding, the polymerase clears the promoter (parameter k_c) and moves along the DNA (parameter k_t). The factor σ is released during this process. Its concentration being non limiting in the environmental conditions considered in our study, it will not be modeled explicitly. Complexes Y and Y^i describe the elongating RNA polymerase, which adds nucleotides one by one. Addition of a last nucleotide completes the full length mRNA and releases the RNA polymerase. The completed RNA molecule is subject to degradation (parameter k_m). It is used by ribosomes as a template for the synthesis of a new RNA polymerase [1]. In the translation step, the ribosome R forms a complex RRNA$'$ with the free ribosome binding site RNA$'$ on the newly synthesized mRNA. After clearance of the ribosome binding site (parameter k_w), the elongated form of the ribosome (noted X) starts synthesizing the protein: aminoacids carried by tRNAs (tRNA*) are transferred one by one to the nascent protein (parameter $k_t^{'}$) giving the complexes X^1, X^2,...,X^i. Addition of the last aminoacid completes the protein, which is released together with the ribosome. The newly synthesized RNA polymerase is competent to start transcribing its own gene or other cellular genes. It is subject to degradation (parameter k_p).

[1] The process of translation can be initiated from every nascent mRNA as shown in [42]. For simplicity, we suppose in this paper that proteins are synthesized from completed mRNAs only.

$$
\begin{aligned}
\sigma + P + D &\underset{k_+}{\overset{k_-}{\leftrightarrows}} PD \\
PD &\xrightarrow{k_c} Y + D + \sigma \\
Y + Nu &\xrightarrow{k_t} Y^1 \\
Y^1 + Nu &\xrightarrow{k_t} Y^2 \\
&\vdots \\
Y^{L-1} + Nu &\xrightarrow{k_t} P + RNA \\
RNA &\xrightarrow{k_m} \phi \\
R + RNA' &\underset{k'_+}{\overset{k'_-}{\leftrightarrows}} RRNA' \\
RRNA' &\xrightarrow{k_w} X + RNA' \\
X + tRNA^* &\xrightarrow{k'_t} tRNA + X^1 \\
X^1 + tRNA^* &\xrightarrow{k'_t} tRNA + X^2 \\
&\vdots \\
X^{H-1} + tRNA^* &\xrightarrow{k'_t} R + P \\
P &\xrightarrow{k_p} \phi
\end{aligned}
$$

FIGURE 4.2.1: Reaction scheme of the transcription-translation model.

4.2.2 Full equations

Nucleotides and amino acids are supposed to be non limiting, as well as sigma factors, and their concentrations are included in the parameters. In the absence of contradictory information, the free and bound forms of RNA polymerase and mRNA are considered to be degraded at the same rate. Degradation of the bound forms (PD and RRNA$'$) releases the promoter and the ribosome. We consider that the free mRNA represents the mRNA with free ribosome binding site RNA$'$.

Using mass action kinetics laws, we obtain:

$$
\begin{aligned}
\dot{c} &= k_+\, p\, d - k_-\, c - k_c\, c - k_p\, c \\
\dot{d} &= -k_+\, p\, d + k_-\, c + k_c\, c + k_p\, c \\
\dot{p} &= -k_+\, p\, d + k_t\, y^{L-1} + k_-\, c + k_t^{'}\, x^{H-1} - k_p\, p \\
\dot{y} &= k_c\, c - k_t\, y - k_p\, y \\
\dot{y}^1 &= k_t\, y - k_t\, y^1 - k_p\, y^1 \\
\dot{y}^2 &= k_t\, y^1 - k_t\, y^2 - k_p\, y^2 \\
&\vdots \\
\dot{y}^{L-1} &= k_t\, y^{L-2} - k_t\, y^{L-1} - k_p\, y^{L-1} \\
\dot{w} &= k_+^{'}\, r\, m - k_-^{'}\, w - k_w\, w - k_m\, w \\
\dot{m} &= -k_+^{'}\, r\, m + k_-^{'}\, w + k_w\, w + k_t\, y^{L-1} - k_m\, m \\
\dot{r} &= -k_+^{'}\, r\, m + k_-^{'}\, w + k_t^{'}\, x^{H-1} + k_m\, w \\
\dot{x} &= k_w\, w - k_t^{'}\, x \\
\dot{x}^1 &= k_t^{'}\, x - k_t^{'}\, x^1 \\
&\vdots \\
\dot{x}^{H-1} &= k_t^{'}\, x^{H-2} - k_t^{'}\, x^{H-1}
\end{aligned}
\tag{4.2.1}
$$

Where p, d, c, y, y^i, and m are the concentrations of P, D, PD, Y, Y^i and $mRNA$ respectively, and where w, r , x, and x^i are the concentrations of RRNA$'$, R, X, and X^i respectively. L and H are the lengths of the mRNA and the protein, respectively [2].

Remark 4.1. We need to mention that our model remains valid if we consider a constant growth rate of the cell μ. The growth of the cell leads to a dilution of the different species, so we suppose that the growth rate μ is included in all the degradation rate.

If the RNA polymerase bound to the promoter D is degraded with a term $-k_p\, c$, this releases simultaneously the same quantity $k_p\, c$ for d (the promoter is not degraded during the degradation of the PD complex). This means that the mass conservation of the total concentration of the promoter remains valid

$$
\begin{aligned}
&\frac{d}{dt}(c + d) = 0 \\
&d = d_0 - c
\end{aligned}
$$

and we can reduce system (4.2.1) by replacing the three first equations with:

[2] The length of the mRNA L is frequently equal to the gene length, the transcription of one base in the gene give one nucleotide. Similarly, H is equal to $L/3$, because the translation of three combined nucleotides in the mRNA give one amino acid.

$$\begin{aligned}\dot{c} &= k_+\, p\,(d_0 - c) - k_-\, c - k_c\, c - k_p\, c \\ \dot{p} &= -k_+\, p\,(d_0 - c) + k_t\, y^{L-1} + k_-\, c + k_t^{'}\, x^{H-1} - k_p\, p\end{aligned} \tag{4.2.2}$$

The total number of ribosomes is also conserved:

$$\begin{aligned}&\frac{d}{dt}(r + w + x + x^1 + \cdots + x^{H-1}) = 0 \\ &r + w + x + x^1 + \cdots + x^{H-1} = R_0\end{aligned}$$

The lengths of the $mRNA$ (L) is large (up to several thousands), as well as the protein lengths (H) which is large, so the above system (4.2.1) is of high dimension. It is also nonlinear and non monotone, for example $\frac{\partial \dot{m}}{\partial r} = -k_+^{'}\, m$ is negative. This makes its analysis difficult and calls for a simplification of the full system.

4.3 Time-scale reduction (Fast-Slow Behavior)

4.3.1 Parameters values for the coupled transcription translation models of RNA polymerase

Parameter values in Tables (4.1), (4.2) and (4.3) have been carefully determined from classical papers in the literature such as [10].

TABLE 4.1: Values of model parameters (fast scale)

Parameters	Values	Units
k_+	60	$\mu\text{M}^{-1}min^{-1}$
k_-	40	min^{-1}
k_c	15	min^{-1}
k_t	2340	nucleotides.min^{-1}
L	8253	nucleotides
$k_+^{'}$	11	$\mu\text{M}^{-1}min^{-1}$
$k_-^{'}$	100	min^{-1}
k_w	80	min^{-1}
$k_t^{'}$	1258	amino acid.min^{-1}
H	2751	amino acids

TABLE 4.2: Values of model parameters (slow scale)

Parameters	Values	Units
k_p	0.012	min^{-1}
k_m	0.17	min^{-1}

TABLE 4.3: Initial conditions

Initial conditions	Values	Units
d_0	0.000347	μM
R_0	45	μM
z_0	0.5	μM

In Table 4.1, the ratio $\frac{L}{k_t}$, and $\frac{H}{k_t'}$ are constant, see [42]. Taking another gene length L or H will change the value of k_t or k_t' to: $k_t = (2340/8253)L$, and $k_t' = (1258/2751)H$.

The full system (4.2.1) has two different time scales, fast and slow, where k_p and k_m, are small compared to the other parameters in Table (4.1). With these values, the full system can be rather well approximated by a reduced system, using the fact that some variables are faster than others.

In the following we are in a position to apply Tikhonov's Theorem to reduce the full system, this theorem applies to reduce systems of the form:

4.3.2 Separate the full system into "fast" and "slow" variables

We return now to our full system, therefore we chose the following slow variables:

$$z = c + p + y + y^1 + \ldots + y^{L-1} \tag{4.3.1}$$

$$q = m + w \tag{4.3.2}$$

These slow variables represent the total concentration (free and bound form) of the RNA polymerase z and the total concentration of the mRNA with a free and occupied ribosome binding site q (there are also mRNAs bound to elongating ribosomes in X and X^i complexes complexes).

To verify that the evolution of z and q is slow, we scale the variables $y, y^1, ..., y^{L-1}$ with respect to the $mRNA$ length L and the variables $x, x^1, ..., x^{H-1}$ with respect to the protein length H. Consider $\overline{y} = Ly, \overline{y}^i = Ly^i$ and $\overline{x} = Hx, \overline{x^i} = Hx^i$, this gives:

$$
\begin{aligned}
\dot{c} &= k_+\, p\,(d_0 - c) - k_-\, c - k_c\, c - k_p\, c \\
\dot{p} &= -k_+\, p\,(d_0 - c) + \frac{k_t}{L}\,\overline{y}^{L-1} + k_-\, c + \frac{k_t^{'}}{H}\,\overline{x}^{H-1} - k_p\, p \\
\dot{\overline{y}} &= L k_c\, c - k_t\,\overline{y} - k_p\,\overline{y} \\
\dot{\overline{y}}^1 &= k_t\,\overline{y} - k_t\,\overline{y}^1 - k_p\,\overline{y}^1 \\
\dot{\overline{y}}^2 &= k_t\,\overline{y}^1 - k_t\,\overline{y}^2 - k_p\,\overline{y}^2 \\
&\vdots \\
\dot{\overline{y}}^{L-1} &= k_t\,\overline{y}^{L-2} - k_t\,\overline{y}^{L-1} - k_p\,\overline{y}^{L-1} \\
\dot{w} &= k_+^{'}\, r\, m - k_-^{'}\, w - k_w\, w - k_m\, w \\
\dot{m} &= -k_+^{'}\, r\, m + k_-^{'}\, w + k_w\, w + \frac{k_t}{L}\,\overline{y}^{L-1} - k_m\, m \\
\dot{r} &= -k_+^{'}\, r\, m + k_-^{'}\, w + \frac{k_t^{'}}{H}\,\overline{x}^{H-1} + k_m\, w \\
\dot{\overline{x}} &= H k_w\, w - k_t^{'}\,\overline{x} \\
\dot{\overline{x}}^1 &= k_t^{'}\,\overline{x} - k_t^{'}\,\overline{x}^1 \\
&\vdots \\
\dot{\overline{x}}^{H-1} &= k_t^{'}\,\overline{x}^{H-2} - k_t^{'}\,\overline{x}^{H-1}
\end{aligned}
\tag{4.3.3}
$$

where $\frac{k_t}{L}$, $\frac{k_t^{'}}{H}$ are small compared to k_t and $k_t^{'}$ (the bold terms represent the slow evolution parts). So, $z = c + p + \frac{1}{L}(\overline{y} + \overline{y}^1 + \ldots + \overline{y}^{L-1})$, then $\dot{z} = \frac{\mathbf{k_t^{'}}}{\mathbf{H}}\,\overline{x}^{H-1} - \mathbf{k_p}\, z$. Similarly, $q = m + w$ therefore $\dot{q} = \frac{\mathbf{k_t}}{\mathbf{L}}\,\overline{y}^{L-1} - \mathbf{k_m}\, q$.

4.3.3 Fast-Slow subsystems of the full system

Introducing the two new variables z and q, the system (4.2.1) becomes:

$$
\begin{aligned}
\dot{\mathbf{z}} &= k_t^{'}\, x^{h-1} - k_p\, z \\
\dot{\mathbf{q}} &= k_t\, y^{l-1} - k_m\, q \\
\dot{c} &= k_+\, p\,(d_0 - c) - k_-\, c - k_c\, c - \mathbf{k_p}\, \mathbf{c} \\
\dot{p} &= -k_+\, p\,(d_0 - c) + k_t\, y^{L-1} + k_-\, c + \mathbf{k_t^{'}}\, \mathbf{x^{H-1}} - \mathbf{k_p}\, \mathbf{p} \\
\dot{y} &= k_c\, c - k_t\, y - \mathbf{k_p}\, \mathbf{y} \\
\dot{y}^1 &= k_t\, y - k_t\, y^1 - \mathbf{k_p}\, \mathbf{y^1} \\
\dot{y}^2 &= k_t\, y^1 - k_t\, y^2 - \mathbf{k_p}\, \mathbf{y^2} \\
&\vdots \\
\dot{y}^{L-1} &= k_t\, y^{L-2} - k_t\, y^{L-1} - \mathbf{k_p}\, \mathbf{y^{L-1}} \\
\dot{w} &= k_+^{'}\, r\,(q - w) - k_-^{'}\, w - k_w\, w - \mathbf{k_m}\, \mathbf{w} \\
\dot{r} &= -k_+^{'}\, r\,(q - w) + k_-^{'}\, w + k_t^{'}\, x^{h-1} + \mathbf{k_m}\, \mathbf{w} \\
\dot{x} &= k_w\, w - k_t^{'}\, x \\
\dot{x}^1 &= k_t^{'}\, x - k_t^{'}\, x^1 \\
&\vdots \\
\dot{x}^{H-1} &= k_t^{'}\, x^{H-2} - k_t^{'}\, x^{H-1}
\end{aligned}
\tag{4.3.4}
$$

Taking into account the values of parameters above, the dynamics of the system (4.3.4) is similar to the dynamics of the following system:

$$
\begin{aligned}
\dot{z} &= k_t^{'} x^{h-1} - k_p\, z \\
\dot{q} &= k_t\, y^{l-1} - k_m\, q \\
\dot{c} &= k_+\, p\, (d_0 - c) - k_-\, c - k_c\, c \\
\dot{p} &= -k_+\, p\, (d_0 - c) + k_t\, y^{l-1} + k_-\, c \\
\dot{y} &= k_c\, c - k_t\, y \\
\dot{y}^1 &= k_t\, y - k_t\, y^1 \\
\dot{y}^2 &= k_t\, y^1 - k_t\, y^2 \\
&\vdots \\
\dot{y}^{l-1} &= k_t\, y^{l-2} - k_t\, y^{l-1} \\
\dot{w} &= k_+^{'} r\, (q - w) - k_-^{'} w - k_w\, w \\
\dot{r} &= -k_+^{'} r\, (q - w) + k_-^{'} w + k_t^{'} x^{h-1} \\
\dot{x} &= k_w\, w - k_t^{'} x \\
\dot{x}^1 &= k_t^{'} x - k_t^{'} x^1 \\
&\vdots \\
\dot{x}^{h-1} &= k_t^{'} x^{h-2} - k_t^{'} x^{h-1} \\
z &= c + p + y + y^1 + \ldots + y^{L-1}
\end{aligned}
\tag{4.3.5}
$$

We can see that it is difficult to compute the fast variables as a function of the slow variable z, therefore we include the algebraic equation $z = c + p + y + y^1 + \ldots + y^{L-1}$, which makes a link between the slow subsystem with z and the fast part with $(c, p, y, y^1, \ldots, y^{L-1})$.

So, under the parameter values in Tables (4.1), (4.2), if z changes (slowly) as:

$$
\dot{z} = k_t^{'} x^{h-1} - k_p\, z \tag{4.3.6}
$$

$c, p, y, y^1, \ldots, y^{L-1}$ will follow the fast scale dynamics:

$$
\begin{aligned}
\dot{c} &= k_+\, p\,(d_0 - c) - k_-\, c - k_c\, c \\
\dot{p} &= -k_+\, p\,(d_0 - c) + k_t\, y^{l-1} + k_-\, c \\
\dot{y} &= k_c\, c - k_t\, y \\
\dot{y}^1 &= k_t\, y - k_t\, y^1 \\
\dot{y}^2 &= k_t\, y^1 - k_t\, y^2 \\
&\vdots \\
\dot{y}^{l-1} &= k_t\, y^{l-2} - k_t\, y^{l-1}
\end{aligned}
\tag{4.3.7}
$$

and should conserve the hyperplane $z = c + p + y + y^1 + \ldots + y^{L-1}$.

We can reduce the full system if we suppose that the fast subsystem (4.3.7) with variables $c, p, y, y^1, \ldots, y^{L-1}$ converge to a steady state while z changes slowly. But this is valid only if this fast subsystem (4.3.7) has a unique equilibrium which is globally stable, as presented above in the conditions for applying Tikhonov's theorem.

Similarly, we have:

$$
\dot{q} = k_t\, y^{l-1} - k_m\, q \tag{4.3.8}
$$

Which varies slowly, and $w, r, x, x^1, \ldots, x^{H-1}$ varies very fast with this dynamics:

$$
\begin{aligned}
\dot{w} &= k_+^{'}\, r\,(q - w) - k_-^{'}\, w - k_w\, w \\
\dot{r} &= -k_+^{'}\, r\,(q - w) + k_-^{'}\, w + k_t^{'}\, x^{h-1} \\
\dot{x} &= k_w\, w - k_t^{'}\, x \\
\dot{x}^1 &= k_t^{'}\, x - k_t^{'}\, x^1 \\
&\vdots \\
\dot{x}^{h-1} &= k_t^{'}\, x^{h-2} - k_t^{'}\, x^{h-1}
\end{aligned}
\tag{4.3.9}
$$

and should conserve the hyperplane $R_0 = w + r + x + x^1 + \ldots + x^{h-1}$.

Thus, if q changes slowly following dynamics (4.3.8), then $w, r, x, x^1, \ldots, x^{L-1}$ will change fastly with dynamics (4.3.9) and the hyperplane $R_0 = w+r+x+x^1+\ldots+x^{h-1}$. Similarly, we can reduce the full system if we suppose also that the fast subsystem (4.3.9) with the variables $w, r, x, x^1, \ldots, x^{L-1}$ converges to a quasi steady state; but we have also to check conditions of Tikhonov's theorem for the fast subsystem (4.3.9).

4.4 Checking conditions of Tikhonov's Theorem for the fast subsystems

We have first to study the existence and uniqueness of the steady state and the global asymptotic stability of the subsystems (4.3.7) and (4.3.9) which represent the fast part of the full system:

The first fast subsystem (4.3.7), represent exactly the "closed" transcription model which is already has been studied in the previous chapter. We proved that for each hyperplane $H_z = \{(c, p, y, y^1, \ldots, y^{l-1}) \in \Re^{l+2} : \{c + p + y + y^1 + \ldots$
$+y^{l-1} = z > 0\}$, the system (4.3.7) has a unique equilibrium on this invariant hyperplane H_z, which is globally asymptotically stable. The second fast subsystem (4.3.9) represent also the "closed" translation model investigated above in the previous chapter, we remind that the study of the equilibrium of this subsystem (4.3.9) leads to the same results: it has a unique equilibrium on each invariant hyperplane $G = \{(w, r, x, x^1, \ldots x^{h-1}) \in \Re^{h+2} :$ $w + r + x + x^1 + \ldots + x^{h-1} = R_0\}$, and which is globally asymptotically stable.

4.5 Applying Tikhonov's Theorem

We return now to the full system (4.3.5)

- The slow subsystem is given by

$$\begin{aligned} \dot{z} &= k_t^{'} x^{h-1} - k_p z \\ \dot{q} &= k_t y^{l-1} - k_m q \end{aligned} \tag{4.5.1}$$

- For the fast part we obtain the flowing equations for the steady state:

$$\begin{aligned} y^{L-1} = \ldots = y^1 = y &= \frac{k_c}{k_t} c \\ c &= d_0 \frac{p}{p + k_1}, \\ k_1 &= \frac{k_- + k_c}{k_+} \\ x^{H-1} = \ldots = x^1 = x &= \frac{k_w}{k_t^{'}} w \\ w &= \frac{q r}{r + k_2} \\ k_2 &= \frac{k_-^{'} + k_w}{k_+^{'}} \end{aligned} \tag{4.5.2}$$

After proving above that the subsystems have a unique equilibrium which is globally stable, so we can say now that if q, or z change slowly following the dynamics (5.3.2), the fast subsystems (4.3.7) and (4.3.9) will converge to the equilibrium (5.3.1). So, replacing $c, y, y^1, \ldots, y^{L-1}$ with the equation of the equilibrium, which are as a function of p, replacing also $w, x, x^1, \ldots, x^{H-1}$ with the the equilibrium which are as a function of r, the conservation:

$$\begin{aligned} z &= c + p + y + y^1 + \ldots + y^{L-1} \\ R_0 &= w + r + x + x^1 + \ldots + x^{h-1} \end{aligned}$$

becomes:

$$\begin{aligned} p + \frac{d_0\, p}{p + k_1} + l\, \frac{k_c}{k_t}\, \frac{d_0\, p}{p + k_1} &= z \\ r + \frac{q\, r}{r + k_2} + h \frac{k_w}{k'_t}\, \frac{q\, r}{r + k_2} &= R_0 \end{aligned} \tag{4.5.3}$$

let's take this function:

$$M(p) = p + \frac{d_0\, p}{p + k_1} + l\, \frac{k_c}{k_t}\, \frac{d_0\, p}{p + k_1} \tag{4.5.4}$$

This function (4.5.4) as a function of p is an increasing function which tends to 0 if p tends to 0 and tends to $+\infty$ if p tends to $+\infty$, thus for all fixed positive value of $z > 0$: $M(p) = z$ has one positive (unique) solution p^*, this what we have already known before using the theorem (3.1). The theorem (3.1) allowed us to conclude that this equilibrium p^* is globally stable with respect to each positive value of z.

Similarly, for this function:

$$G(r) = r + \frac{q\, r}{r + k_2} + h \frac{k_w}{k'_t}\, \frac{q\, r}{r + k_2} \tag{4.5.5}$$

as a function of r, which for a fixed value of q is quite similar to the function (4.5.4), so we can say also that for all fixed positives values of q then $G(r) = R_0$ has a unique positive solution r^*, which is also globally stable with respect to each positive value of q.

4.6 The Reduced System

Applying the quasi-steady state approximation to the fast variables and keeping only the slow ones (replacing x^{h-1} and y^{l-1} by the equations of the equilibrium of the fast subsystems), allows to write the reduced system:

$$\begin{aligned} \dot{z} &= k_w \frac{q\, r(q)}{r(q)+k_2} - k_p\, z \\ \dot{q} &= k_c d_0 \frac{p(z)}{p(z)+k_1} - k_m\, q \end{aligned} \tag{4.6.1}$$

In this system $p(z)$ and $r(q)$ are calculated from the following algebraic equations (4.6.2) and (4.6.3):

$$z = l\,\frac{k_c}{k_t}\, d_0\, \frac{p(z)}{p(z)+k_1} + p(z) + d_0\, \frac{p(z)}{p(z)+k_1} \tag{4.6.2}$$

$$R_0 = h\frac{k_w}{k_t'}\frac{q\, r(q)}{r(q)+k_2} + r(q) + \frac{q\, r(q)}{r(q)+k_2} \tag{4.6.3}$$

These algebraic equations have only one positive solution $p(z)$ and $r(q)$, which is shown above.

4.7 Simulations of the full and the reduced system

We performed simulations to show the similarity of the full and reduced systems. The dashed line represents the evolutions of the reduced system, and the full line shows the evolutions of the complete system. The total concentration of the polymerase, and the concentration of the mRNA are represented in figures ((4.7.1),(4.7.2)). Their behaviours are rather similar, the full system oscillates a little bit at the beginning and the reduced system does not oscillate. Of course, the quality of reduction depends on the parameter values. In the literature, RNA polymerase levels should be much lower (in a range of $5-10\mu M$), but that for the purpose of this chapter it is not important to quantitatively reproduce the observed behavior.

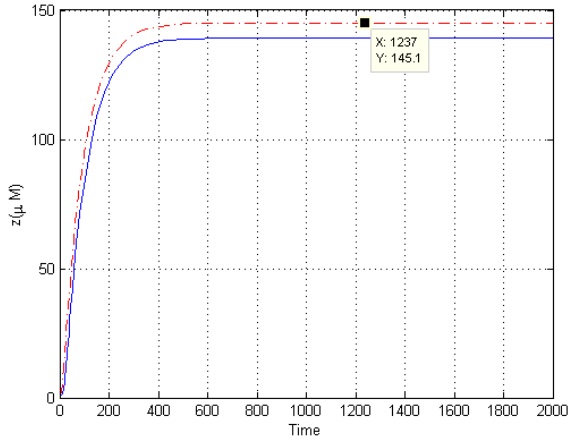

FIGURE 4.7.1: The evolutions of the total concentration of the polymerase z in the reduced system and in the complete system, with initial conditions: $p_0 = z_0 = 0.5$, and with taking $l = 100$, $h = 33$.

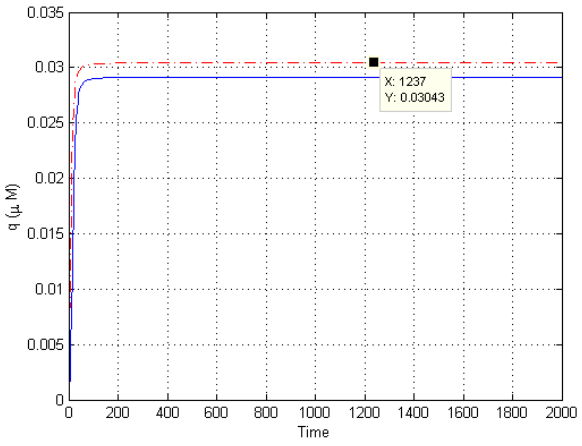

FIGURE 4.7.2: The evolutions of the concentration of the mRNA q in the reduced system and in the complete system, with $p_0 = z_0 = 0.5$, and $l = 100$, $h = 33$.

4.8 Dynamical study of the Reduced System

4.8.1 Equilibria of the reduced system

At the equilibrium we get:

$$\begin{aligned} z &= \frac{k_w}{k_p} \frac{q\, r(q)}{r(q) + k_2} \\ q &= \frac{k_c d_0}{k_m} \frac{p(z)}{p(z) + k_1} \end{aligned} \tag{4.8.1}$$

We therefore obtain z as a function of q, and q as a function of z.

To simplify the algebraic equations, we take :

$$\begin{aligned} \gamma &= (L\,\frac{k_c}{k_t} + 1)\,d_0 \\ \lambda &= H\frac{k_w}{k_t'} + 1. \end{aligned} \tag{4.8.2}$$

The algebraic equations become:

$$\begin{aligned} z &= \gamma\frac{p(z)}{p(z)+k_1} + p(z) \\ R_0 &= \lambda\frac{q\,r(q)}{r(q)+k_2} + r(q). \end{aligned} \tag{4.8.3}$$

From these algebraic equations, we have also at the equilibrium:

$$\begin{cases} \dfrac{p(z)}{p(z)+k_1} = \dfrac{1}{\gamma}(z-p(z)) \\ \dfrac{q\,r(q)}{r(q)+k_2} = \dfrac{1}{\lambda}(R_0-r(q)) \end{cases} \tag{4.8.4}$$

By replacing (4.8.4) in (4.8.1), we get the following equations for the equilibrium:

$$\begin{aligned} z &= \frac{k_w}{k_p\lambda}(R_0-r(q)) \\ q &= \frac{k_c d_0}{k_m\gamma}\,(z-p(z)). \end{aligned} \tag{4.8.5}$$

Consider the functions:

$$\begin{aligned} \xi(q) = z &= \frac{k_w}{k_p}\frac{q\,r(q)}{r(q)+k_2} \\ &= \frac{k_w}{k_p\lambda}(R_0-r(q)) \end{aligned} \tag{4.8.6}$$

and,

$$\begin{aligned} \varphi(z) = q &= \frac{k_c d_0}{k_m}\,\frac{p(z)}{p(z)+k_1} \\ &= \frac{k_c d_0}{k_m\gamma}\,(z-p(z)) \end{aligned} \tag{4.8.7}$$

First we can notice that $\varphi(z)$ is bounded by:

$$\varphi(z) < \frac{k_c d_0}{k_m} \tag{4.8.8}$$

because $\frac{p(z)}{p(z)+k_1} < 1$.

Similarly, $r(q) = R_0 - \frac{k_p \lambda}{k_w}\xi(q)$, should remain positive, which leads to:

$$\xi(q) < \frac{k_w R_0}{k_p \lambda} \tag{4.8.9}$$

Therefore, the equilibria should respect the constraints (4.8.8) and (4.8.9).

To determine the equilibria we have to study the intersections of the two above functions $\xi(q)$ and $\varphi(z)$. We will first study $p(z)$ and $r(q)$ from the algebraic equations (5.4.4). If we derive the first equation with respect to z, and the second with respect to q, we obtain:

$$\begin{aligned} 1 &= \gamma \frac{k_1}{(p(z)+k_1)^2}\frac{\partial p(z)}{\partial z} + \frac{\partial p(z)}{\partial z} \\ 0 &= \lambda \frac{r(q)}{r(q)+k_2} + \lambda q \frac{k_2}{(r(q)+k_2)^2}\frac{\partial r(q)}{\partial q} + \frac{\partial r(q)}{\partial q}. \end{aligned} \tag{4.8.10}$$

We have thus:

$$\begin{aligned} \frac{\partial p(z)}{\partial z} &= \frac{(p(z)+k_1)^2}{\gamma k_1 + (p(z)+k_1)^2} \\ \frac{\partial r(q)}{\partial q} &= -\frac{\lambda \frac{r(q)}{r(q)+k_2}}{\lambda q \frac{k_2}{(r(q)+k_2)^2} + 1}. \end{aligned} \tag{4.8.11}$$

$p(z)$ is positive and increasing, with $p(0) = 0$ and $p(z) \approx z$ when z is large. Similarly, $r(q)$ is positive and decreasing, with $r(0) = R_0$ and $r(q)$ tends toward 0 for large q.

If we derive again equations (5.5.12), the first equation with respect to z, and the second with respect to q, we obtain:

$$\begin{aligned} \frac{\partial^2 p(z)}{\partial z^2} &= \gamma \frac{2k_1}{(p(z)+k_1)^3}\frac{\partial p(z)}{\partial z}^2 \\ (1 + \lambda q \frac{k_2}{(r(q)+k_2)^2})\frac{\partial^2 r(q)}{\partial q^2} &= -2\lambda \frac{k_2}{(r(q)+k_2)^2}\frac{\partial r(q)}{\partial q} + \lambda q \frac{2k_2}{(r(q)+k_2)^3}\frac{\partial r(q)}{\partial q}^2. \end{aligned} \tag{4.8.12}$$

Thus, $\frac{\partial^2 p(z)}{\partial z^2}$ and $\frac{\partial^2 r(q)}{\partial q^2}$ are positive, and we conclude that $p(z)$ and $r(q)$ are also convex. The same results ($p(z)$ is positive, increasing and convex, $r(q)$ is positive, decreasing and convex) can be obtained from the study of the positive analytic solutions of the algebraic equations, which are quadratic equations (4.8.13):

$$\begin{aligned} p(z) &= \frac{1}{2}(z - \gamma - k_1 + \sqrt{z^2 - 2\gamma z + 2k_1 z + \gamma^2 + 2\gamma k_1 + k_1^2}) \\ r(q) &= \frac{1}{2}(R_0 - \lambda q - k_2 + \sqrt{R_0^2 - 2\lambda q R_0 + 2k_2 R_0 + \lambda^2 q^2 + 2\lambda k_2 q + k_2^2}). \end{aligned} \tag{4.8.13}$$

Now, we come back to the study of the functions $\xi(q)$ and $\varphi(z)$. The derivative of $\xi(q)$ is:

$$\xi'(q) = -\frac{k_w}{k_p \lambda} \frac{\partial r(q)}{\partial q} \tag{4.8.14}$$

which is positive because $\frac{\partial r(q)}{\partial q}$ is negative. We also have:

$$\xi''(q) = -\frac{k_w}{k_p \lambda} \frac{\partial^2 r(q)}{\partial q^2} \tag{4.8.15}$$

which is negative, because $\frac{\partial^2 r(q)}{\partial q^2}$ is positive.

The function $\xi(q)$ is therefore an increasing, positive and concave function, and it is bounded ($\xi(q) < \frac{k_w R_0}{k_p \lambda}$).

Similarly, for $\varphi(z)$, we have:

$$\varphi'(z) = \frac{k_c d_0}{k_m} \frac{k_1}{(p(z) + k_1)^2} \frac{\partial p(z)}{\partial z} = \frac{k_c d_0}{k_m \gamma} (1 - \frac{\partial p(z)}{\partial z})$$

which is positive, because $\frac{\partial p(z)}{\partial z}$ is positive, or because $\frac{\partial p(z)}{\partial z} < 1$, see the equation (4.8.11). We also have:

$$\varphi''(z) = -\frac{k_c d_0}{k_m \gamma} \frac{\partial^2 p(z)}{\partial z^2} \tag{4.8.16}$$

which is negative, because $\frac{\partial^2 p(z)}{\partial z^2}$ is positive.

So, $\varphi(z)$ is an increasing, positive, concave and it is also bounded ($\varphi(z) < \frac{k_c d_0}{k_m}$).

If we draw the phase space (q, z), two cases are possible (see, Figures (4.8.1) and (4.8.2)): either (0,0) is the unique equilibrium, or there exists another unique, positive equilibrium

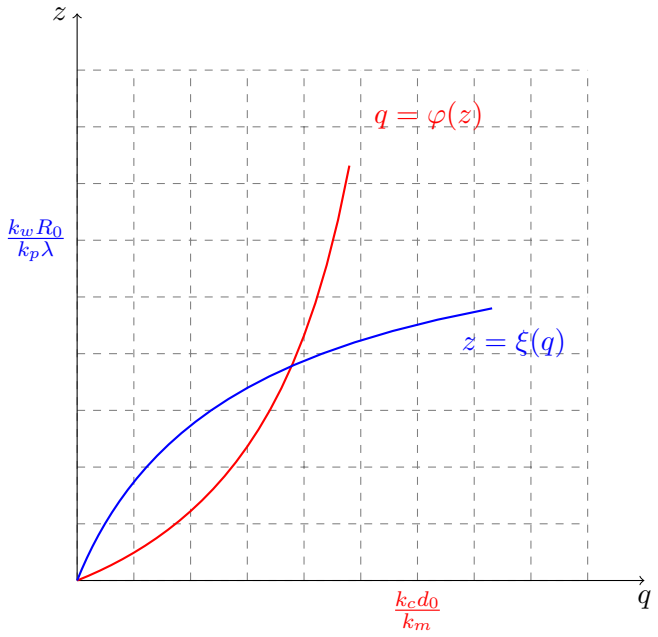

FIGURE 4.8.1: The phase space (q, z) in the case that two equilibria exist: $(0, 0)$ and another one which is strictly positive (z^*, q^*) under the condition $\xi'(q)|_{q=0} > \frac{1}{\varphi'(z)|_{z=0}}$.

(the point (0,0) is always an equilibrium for this system). The alternative between these two cases depends on the slope at the origin (0,0).

Proposition 4.2.

- *If :*

$$\begin{aligned} &\xi'(q)\big|_{q=0} > \frac{1}{\varphi'(z)|_{z=0}} \\ &\Rightarrow \frac{k_w}{k_p}\frac{R_0}{R_0+k_2} > \frac{k_m}{k_c d_0}(\gamma + k_1) \\ &\Rightarrow k_m k_p < k_w \frac{k_c d_0}{\gamma + k_1}\frac{R_0}{R_0+k_2} \end{aligned} \tag{4.8.17}$$

then there exist two equilibria which are $(0, 0)$*, and a unique, positive* (z^*, q^*)

- *If:*

$$\begin{aligned} &\xi'(q)\big|_{q=0} < \frac{1}{\varphi'(z)|_{z=0}} \\ &\Rightarrow k_m k_p > k_w \frac{k_c d_0}{\gamma + k_1}\frac{R_0}{R_0+k_2} \end{aligned} \tag{4.8.18}$$

then there exists only one equilibrium for the system which is $(0, 0)$*.*

4.8.2 Study of the stability of equilibria

In this section, we study the global stability for each case.

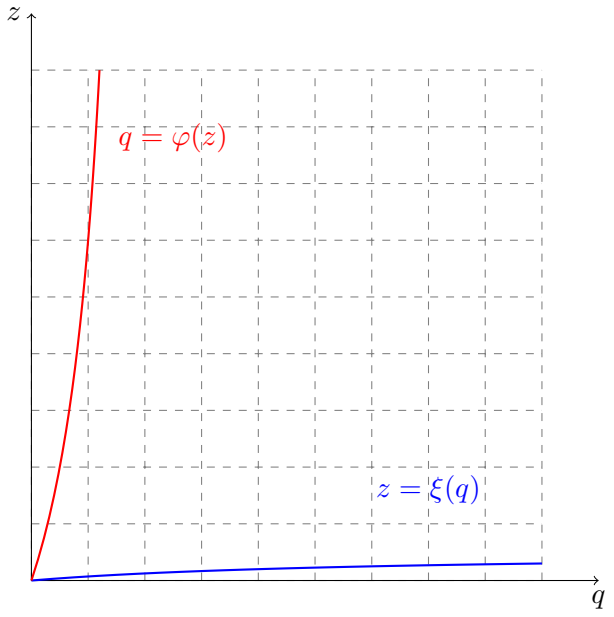

FIGURE 4.8.2: The phase space (q, z) in the case that one equilibrium exists which is $(0, 0)$ if $\xi'(q)|_{q=0} < \frac{1}{\varphi'(z)|_{z=0}}$.

First, the Jacobian matrix of the reduced system (4.6.1) is:

$$J(x) = \begin{pmatrix} -k_p & k_p \frac{\partial \xi(q)}{\partial q} \\ k_m \frac{\partial \varphi(z)}{\partial z} & -k_m \end{pmatrix}. \tag{4.8.19}$$

We have already proved that $\xi(q)$ is an increasing function of q and $\varphi(z)$ is an increasing function of z. Therefore the Jacobian matrix $J(z, q)$ is off-diagonal positive, which means that the reduced system (4.6.1) is monotone [58]. Therefore, for the two cases, if we can find a point in the state space greater than the equilibrium point (z^*, q^*) with respect to the usual partial ordering where all the derivatives are non-positive, and similarly, a point smaller than the equilibrium where the derivatives are non-negative, then all the trajectories initiating in this rectangle converge towards the equilibrium. For more details about these techniques from monotone systems and this theorem for the equilibrium, see the Appendix A.1.

Remark 4.3. The Jacobian matrix of the full system (4.2.1), is with off-diagonal non positive elements, the full system was non monotone. Using Tikhonov's Theorem leads to a monotone reduced system, therefore we were able to study and analyze the reduced model (which has the same behavior as the full model) using monotone system theory, which allowed us to have conclusions for the full system.

4.8.2.1 Stability of the equilibrium $(0,0)$ in the case that it is the unique equilibrium of the system

Proposition 4.4. *If* $k_m k_p - k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2} > 0$ *then*

- $(0,0)$ *is the unique equilibrium of the system and it is globally stable in the non-negative orthant.*

Proof. The Jacobian matrix at the equilibrium is:

$$\begin{aligned} J(x) &= \begin{pmatrix} -k_p & k_p \xi'(0) \\ k_m \varphi'(0) & -k_m \end{pmatrix} \\ &= \begin{pmatrix} -k_p & k_w \frac{R_0}{R_0 + k_2} \\ \frac{k_c d_0}{\gamma + k_1} & -k_m \end{pmatrix}. \end{aligned} \tag{4.8.20}$$

Thus, the equilibrium $(0,0)$ in this case is locally stable, because the trace is negative and the determinant is positive ($k_m k_p - k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2} > 0$ is the condition (4.8.18) to have only $(0,0)$ as an equilibrium of the system).

When the variables (z, q) are large, the vector field

$$\begin{aligned} \dot{z} &= k_w \frac{q\, r(q)}{r(q) + k_2} - k_p\, z \\ \dot{q} &= k_c d_0\, \frac{p(z)}{p(z) + k_1} - k_m\, q \end{aligned} \tag{4.8.21}$$

is negative, because:

- $\frac{p(z)}{p(z)+k_1}$ is bounded, so $\dot{q} = k_c d_0 \frac{p(z)}{p(z)+k_1} - k_m\, q$ will tends to $-\infty$ if q tends to $+\infty$, so $\dot{q}$ is non-positive.
- We have proved above that $\xi(q)$ is also bounded, therefore, if z tends to $+\infty$, $\dot{z} = k_w \frac{q\, r(q)}{r(q)+k_2} - k_p\, z$, will tends to $-\infty$, which is negative.

Thus, if we choose a point arbitrarily large enough, we can apply the above techniques from monotone systems, to obtain that $(0,0)$ is globally stable in the nonnegative orthant.

□

4.8.2.2 Stability in the case that two equilibria exist, which are $(0,0)$, and a unique, positive (z^*, q^*)

Proposition 4.5. *If* $k_m k_p - k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2} < 0$ *then*

- $(0,0)$ *is unstable.*
- *The positive equilibrium* (z^*, q^*) *is globally stable in the positive orthant.*

Proof.

- For the equilibrium $(0,0)$, we can easily notice that the determinant of the Jacobian matrix near $(0,0)$ is negative ($k_m k_p < k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2}$ is the condition (4.8.17) to have two equilibria for the system), thus $(0,0)$ is an unstable saddle point.
- For the positive equilibrium (z^*, q^*), we have the characteristic polynomial is the following:

$$\lambda^2 + (k_p + k_m)\lambda + k_p k_m - k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2} = 0$$

Thus, this gives the following eigenvalues:

$$\lambda_{01} = \frac{1}{2}(-(k_p + k_m) + \sqrt{(k_p + k_m)^2 + 4(k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2} - k_p k_m)})$$
$$\lambda_{02} = \frac{1}{2}(-(k_p + k_m) - \sqrt{(k_p + k_m)^2 + 4(k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2} - k_p k_m)})$$

Thus, if $k_m k_p - k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2} < 0$, then λ_{01} is a positive real eigenvalue, λ_{02} is a negative real or complex eigenvalue.

For the positive eigenvalue λ_{01}, the positive eigenvector is given by:

$$\begin{pmatrix} -k_p & k_w \frac{R_0}{R_0 + k_2} \\ \frac{k_c d_0}{\gamma + k_1} & -k_m \end{pmatrix} \begin{pmatrix} z \\ q \end{pmatrix} = \lambda_{01} \begin{pmatrix} z \\ q \end{pmatrix} \tag{4.8.22}$$

We know from the Perron-Frobenius theorem (B.2) in the Appendix for Metzler matrices (all the elements outside the main diagonal are nonnegative), that there exists a point near the equilibrium $(0,0)$ where the vector field is nonnegative, and corresponds to the real positive eigenvalue λ_{01}.

We also know that the vector field is non-positive if (z, q) are large, see above (4.8.21).

We apply the above techniques from monotone systems theory, to conclude that the unique positive equilibrium (z^*, q^*) is globally stable for the positive orthant.

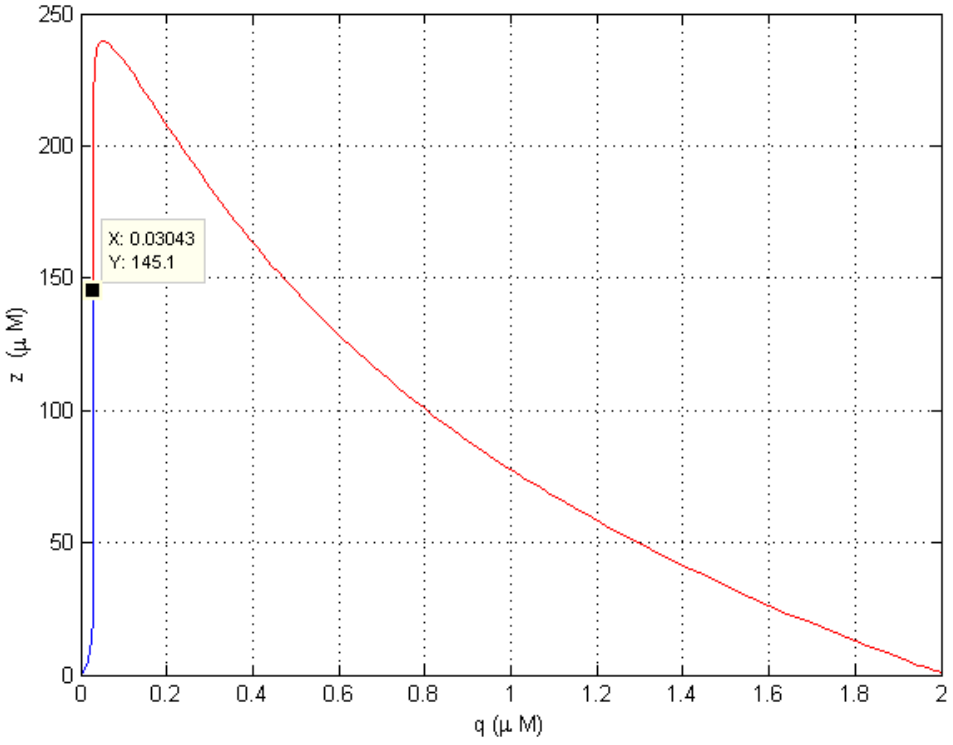

FIGURE 4.8.3: Simulation taking the parameter values above in the tables. This corresponds to the case that two equilibria exist which are $(0, 0)$, and $(0.03045, 145.1)$ which is globally stable in the positive orthant.

□

In summary, the mathematical study of this system gives the following results: the system is monotone [58], with one positive loop, and the solutions are bounded. It has either a single stable equilibrium in $(0, 0)$ or two equilibria, one in zero (unstable) and another stable one (z^*, q^*). The stability or instability of the zero equilibrium depends on the values of the parameters, as shown by computing the eigenvalues of the Jacobian matrix. The global behaviour result is given by monotone systems theory.

The simulations initiating from two different initial conditions of the reduced system are shown in Figure (4.8.3). We observe that two trajectories from two different initial conditions $(q_{01}, z_{01}) = (0, 0.5)$ and $(q_{02}, z_{02}) = (2, 0.8)$ converge to the positive equilibrium.

Remark 4.6. We notice that the time for simulations of the reduced system is very short compared to the simulation of the full system.

4.9 Generalization with qualitative functions

The behaviour of the two rational functions $\xi(q)$ and $\varphi(z)$ are of Michaelis-Menten type: they are positive, strictly increasing, concave and also bounded. Their analytical forms are obtained by the quasi-steady-state approximation. An even more general model can be obtained, if we consider only qualitative classes of functions like $\xi(q)$ and $\varphi(z)$ having the following properties: positive, increasing, concave and bounded. For example if we consider that $M_1(p) = \frac{p}{p+k_1}$,$M_2(r) = \frac{r}{r+k_2}$, a general model of the reduced model in the section (06) is:

$$\begin{aligned} z &= \gamma M_1(p(z)) + p(z) \\ R_0 &= \lambda q M_2(r(q)) + r(q) \\ \dot{z} &= k_w q M_2(r(q)) - k_p\, z \\ \dot{q} &= k_c d_0\, M_1(p(z)) - k_m\, q \end{aligned}$$

we notice, $M_1(p(z) = 0) = 0$, then $p(z = 0) = 0$ and $M_1(z = 0) = 0$. If we derive the first equation of the algebraic equations with respect to z, we obtain:

$$1 = \gamma M_1'(p) p'(z) + p'(z)$$

$M_1'(p)$ should be positive to have a unique positive solution of the algebraic equation, then $p'(z) = \frac{1}{\gamma M_1'(p)+1}$ is positive and we conclude also that $p(z)$ is increasing and that $p'(z) < 1$ ($\gamma M_1'(p)p'(z) > 0$). Differentiating one more time gives:

$$0 = \gamma M_1''(p)(p'(z))^2 + \gamma M_1'(p(z))p''(z) + p''(z)$$

which proves that $p(z)$ is convex.To obtain the monotonicity of the system, we check that $M_1'(p(z))\, p'(z)$ is positive, and to check concavity, we write $M_1(p(z)) = \frac{z-p(z)}{\gamma}$ where it can be seen that $M_1(p(z))$ is concave because $p(z)$ is convex. The proofs are similar for $r(q)$ and $M_2(r(q))$.

Thus, a more general model can be obtained, if we consider only qualitative classes of functions having the following properties: positive, increasing, concave and bounded. The general model is

$$\begin{aligned} \dot{z} &= f(q) - k_z\, z \\ \dot{q} &= g(z) - k_q\, q \\ z &= \alpha g(z) + p(z) \\ R_0 &= \beta f(q) + r(q). \end{aligned} \tag{4.9.1}$$

where $f(q), g(z)$ are positive, strictly increasing, concave and also bounded, with ($f(0) = g(0) = 0$).

We have for the equilibria :

$$\begin{aligned} z &= \frac{1}{k_z} f(q) \\ &= \frac{1}{k_z \beta}(R_0 - r(q)) \\ q &= \frac{1}{k_q} g(z) \\ &= \frac{1}{k_q \alpha}\,(z - p(z)) \end{aligned} \tag{4.9.2}$$

Thus, with these qualitative functions (equals to zero for the origin), the following results hold for the equilibria

- either a unique equilibrium (0,0) exists under the following condition:

$$k_z k_q > f'(q)\big|_{q=0}\, g'(z)\big|_{z=0}$$

- or it exists another equilibrium which is the unique equilibrium in the positive orthant under the following condition:

$$k_z k_q < f'(q)\big|_{q=0}\, g'(z)\big|_{z=0}$$

The Jacobian Matrix of the reduced system (5.4.3) is as follows:

$$J(x) = \begin{pmatrix} -k_z & \frac{\partial f(q)}{\partial q} \\ \frac{\partial g(z)}{\partial z} & -k_q \end{pmatrix} \tag{4.9.3}$$

Therefore, we can apply the above techniques (monotone systems and concavity) to obtain the same results:

- either $(0, 0)$ is globally stable if it is locally stable.

- or $(0,0)$ is locally unstable, and there exists a unique positive equilibrium (z^*, q^*), which is globally stable for the positive orthant.

4.10 Generalization: stability of monotone system with a decreasing Jacobian matrix

The same results hold for a more general system with n dimensions. We are now in a position to use the theorem of monotone system with a decreasing Jacobian matrix. Roughly, this theorem (see Appendix) is a generalization to the dimension n of the elementary fixed point theorem in one dimension for a positive increasing concave function F such that $F(0) = 0$: either 0 is the only fixed point and is globally stable for the iteration $x_{n+1} = F(x_n)$, or there is another unique positive fixed point, which is globally stable in the positive orthant for the same iteration. We give the theorem in the form of [55].

To apply for example this theorem to our reduced model, we have to check the assumptions (remark that these assumptions are all qualitative): firstly, the solutions are bounded, as shown above. The Jacobian matrix is:

$$J(z,q) = \begin{pmatrix} -k_p & k_p \frac{\partial \xi(q)}{\partial q} \\ k_m \frac{\partial \varphi(z)}{\partial z} & -k_m \end{pmatrix} \tag{4.10.1}$$

Because of the properties of functions $\xi(q), \varphi(z)$ studied above, we conclude that J is off-diagonal positive, and moreover irreducible in the positive orthant. Moreover, $\xi(q), \varphi(z)$ are strictly concave, and therefore the Jacobian matrix is decreasing. We can therefore applied this theorem and the same results as previous hold:

- either $(0,0)$ is globally stable if it is locally stable under the following condition:

$$\xi'(q)\big|_{q=0}\, \varphi'(z)\big|_{z=0} < 1 \Rightarrow k_m k_p > k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2}.$$

- or $(0,0)$ is locally unstable, and there exists a unique positive equilibrium (z^*, q^*), which is globally stable for the positive orthant under the following condition:

$$\xi'(q)\big|_{q=0}\, \varphi'(z)\big|_{z=0} > 1 \Rightarrow k_m k_p < k_w \frac{k_c d_0}{\gamma + k_1} \frac{R_0}{R_0 + k_2}.$$

The local stability or non stability of the trivial equilibrium is the unique condition for (respectively) the global stability of the trivial equilibrium or for the global stability of

a unique positive equilibrium. A local computation of the stability of the Jacobian at $(0,0)$ therefore gives all is needed to describe the global behavior of the whole system.

Example 4.1. *Applying this theorem for a different degradation*

In the above model, we supposed that all the forms of polymerase are degraded at the same rate. This assumption could be changed, for example by supposing a degradation of the free form of the polymerase only, and no degradation for the bound forms. The new equations are:

$$\begin{aligned} z &= \gamma \frac{p}{p+k_1} + p \\ R_0 &= \lambda \frac{q\, r}{r+k_2} + r \\ \dot{z} &= k_w \frac{q\, r(q)}{r(q)+k_2} - k_p\, p(z) \\ \dot{q} &= k_c d_0 \frac{p(z)}{p(z)+k_1} - k_m \left(q - \frac{q\, r(q)}{r(q)+k_2}\right) \end{aligned}$$

The conservation equations are the same, but the diagonal elements of the Jacobian matrix change: yet, it is easy to verify that the matrix remains decreasing; for example, the first term $-k_p\, p(z)$ is decreasing and concave because $p(z)$ is increasing and convex. The term $-k_m \left(q - \frac{q\, r(q)}{r(q)+k_2}\right) = -k_m q(\frac{k_2}{r(q)+k_2})$ is decreasing, because $r(q)$ is decreasing. This term is also concave, because $-k_m \left(q - \frac{q\, r(q)}{r(q)+k_2}\right) = -k_m(q - \frac{R_0 - r(q)}{\lambda})$ and $r(q)$ is convex. The same result as above therefore holds for global stability. The new Jacobian matrix at $(0,0)$ is:

$$J(0,0) = \begin{pmatrix} -k_p \frac{k_1}{\gamma+k_1} & k_w \frac{R_0}{R_0+k_2} \\ \frac{k_c d_0}{\gamma+k_1} & -k_m(1 - \frac{R_0}{R_0+k_2}) \end{pmatrix} \tag{4.10.2}$$

Now, global stability only depends on the local stability at $(0,0)$ with the new inequality (for local stability of the trivial equilibrium):

$$k_p k_m > k_w \frac{k_c d_0 R_0}{k_1 k_2}$$

Remark 4.7. This approach is interesting because we do not have now to compute explicitly the values of the equilibria, which can be very difficult.

4.11 Conclusions

Several interesting conclusions can be made from the study of this system. For example, computations lead to the fact that, if R_0 is large (many ribosomes), the zero equilibrium

is unstable; if R_0 is small, the zero equilibrium is globally stable, and everything goes to zero.

These results are in agreement with several biological observations on the adaptation of living organisms to their environment. For instance, in the case of bacteria, the zero equilibrium corresponds to the situation of cells whose growth is arrested by harmful environmental conditions. Translation is halted in these cells, through an arrest of ribosome synthesis and the inactivation of the remaining ribosomes [54, 69]. As a consequence, the intracellular concentration of active ribosomes decreases, which lowers the concentration of RNA polymerase. The essential cell components can no longer be synthesized; cells eventually die if the ribosomes and the RNA polymerase remains at so low concentrations. By contrast, when environmental conditions become favorable again, ribosome synthesis starts immediately and inactivated ribosomes become functional again [54, 69]. The concentration of ribosomes rises in the cell. According to the model, the zero equilibrium becomes unstable in these conditions. The consequence is a rapid accumulation of new pools of RNA polymerase and ribosomes, that are necessary for the cell to synthesize all the precursors needed to grow and divide again.

Our simple loop model could be easily extended so as to include these regulatory mechanisms. Then the reduced system could be included into more general models of the gene expression machinery.

Chapter 5

Stability analysis of a reduced transcription-translation model of RNA polymerase and ribosomes

We consider in this chapter that the total concentration of the ribosome is changing, and couple a ribosome synthesis model with the previous model for polymerase. The system is still monotone, but the Jacobian matrix is not decreasing, therefore we cannot use the same techniques as before in chapter 4 to study the equilibria of the new model. To solve explicitly the equations for the equilibria of this system is difficult, we geometrically prove (using the parameters values of this system) that it exist three equilibria, one at the origin, another one very close to the origin and the last one far from the origin. To simplify we did some approximations and we easily obtain the equations of the equilibria, we study the local stability of each equilibria and we use as previously the same techniques from monotone systems to investigate the global stability of the equilibria. We show that the origin and the far equilibrium are stable, the equilibrium near zero being unstable.

5.1 Introduction

The model studied in the previous chapter is a very simple loop with only polymerase; in fact, it is not isolated from the rest of the cell. The transcription and translation processes are embedded with other regulatory mechanisms. For example, the ribosome is also synthesized by gene expression and can be also degraded (does not remain constant as in the previous chapter). Ribosomes are made of stable RNAs and ribosomal proteins. The stable RNAs are transcribed from the gene and subsequently maturated directly

as a first-order reaction into functional ribosomes. Therefore, stable ribosomal RNAs are produced from one step of transcription and without translation. We consider in this chapter that ribosomes are produced from the transcription of stable RNAs only, because it is considered to be limiting for ribosome production.

The transcription of stable RNAs can be inhibited by ppGpp, which does not influence RNA polymerase, therefore we can have two cases which depends of the concentration of the ppGpp: either the production of the polymerase and ribosomes in the same time (this chapter) or only the production of polymerase (previous chapter).

5.2 A more general model with ribosome synthesis

In the following, we consider that the ribosome is synthesized by some gene expression. The reaction scheme of the ribosome synthesis is given in Figure (5.2.1).

$$
\begin{aligned}
\sigma + P \; + \; D' &\underset{k_+^2}{\overset{k_-^2}{\leftrightarrows}} PD' \\
PD' &\xrightarrow{k_s^2} Y' \; + \; D' \; + \; \sigma \\
Y' \; + \; Nu &\xrightarrow{k_t^2} Y'^1 \\
Y'^1 \; + \; Nu &\xrightarrow{k_t^2} Y'^2 \\
&\vdots \\
Y'^{L^2-1} \; + \; Nu &\xrightarrow{k_t^2} P \; + R \\
R &\xrightarrow{k_r} \phi \\
P &\xrightarrow{k_p} \phi
\end{aligned}
$$

FIGURE 5.2.1: Reaction scheme of the transcription of ribosome

with P represent the RNA polymerase, D' binding site on DNA, complexes Y' and Y'^i describe the moving polymerase which binds to nucleotides along the strand. R the completed stable ribosomal RNAs molecule, which is subject to degradation (parameter k_r).

Therefore, the reaction scheme of the full model with ribosome synthesis is given in Figure (5.2.2).

Nucleotides are supposed to be in excess, and their concentrations are included in the parameters. Therefore, the system becomes as follows:

$$
\begin{aligned}
\sigma + P \ + \ D &\overset{k_-^1}{\underset{k_+^1}{\leftrightarrows}} \ PD \\
PD &\xrightarrow{k_c^1} \ Y \ + \ D \ + \ \sigma \\
Y \ + \ Nu &\xrightarrow{k_t^1} \ Y^1 \\
Y^1 \ + \ Nu &\xrightarrow{k_t^1} \ Y^2 \\
&\vdots \\
Y^{L^1-1} \ + \ Nu &\xrightarrow{k_t^1} \ P \ + \ RNA \\
RNA &\xrightarrow{k_m} \ \phi \\
\sigma + P \ + \ D' &\overset{k_-^2}{\underset{k_+^2}{\leftrightarrows}} \ PD' \\
PD' &\xrightarrow{k_c^2} \ Y' \ + \ D' \ + \ \sigma \\
Y' \ + \ Nu &\xrightarrow{k_t^2} \ Y'^1 \\
Y'^1 \ + \ Nu &\xrightarrow{k_t^2} \ Y'^2 \\
&\vdots \\
Y'^{L^2-1} \ + \ Nu &\xrightarrow{k_t^2} \ P \ + R \\
R &\xrightarrow{k_r} \ \phi \\
R \ + \ RNA &\overset{k_-'}{\underset{k_+'}{\leftrightarrows}} \ RRNA \\
RRNA &\xrightarrow{k_w} \ X \ + \ RNA \\
X \ + \ tRNA^* &\xrightarrow{k_t'} \ tRNA \ + \ X^1 \\
X^1 \ + \ tRNA^* &\xrightarrow{k_t'} \ tRNA \ + \ X^2 \\
&\vdots \\
X^{H-1} \ + \ tRNA^* &\xrightarrow{k_t'} \ R \ + \ P \\
P &\xrightarrow{k_p} \ \phi
\end{aligned}
$$

FIGURE 5.2.2: Reaction scheme of the transcription-translation model with ribosome synthesis.

$$
\begin{aligned}
\dot{c}_1 &= k_+^1\, p\,(d_0^1 - c_1) - k_-^1\, c_1 - k_c^1\, c_1 - \mathbf{k_p\, c_1} \\
\dot{c}_2 &= k_+^2\, p\,(d_0^2 - c_2) - k_-^2\, c_2 - k_c^2\, c_2 - \mathbf{k_p\, c_2} \\
\dot{p} &= -k_+^1\, p\,(d_0^1 - c_1) + k_t^1\, y_1^{L^1-1} + k_{1-}\, c_1 \\
&\quad - k_+^2\, p\,(d_0^2 - c_2) + k_t^2\, y_2^{L^2-1} + k_-^2\, c_2 + \mathbf{k_t^{'}\, x^{H-1}} - \mathbf{k_p\, p} \\
\dot{y}_1 &= k_c^1\, c_1 - k_t^1\, y_1 - \mathbf{k_p\, y_1} \\
\dot{y}_2 &= k_c^2\, c_2 - k_t^2\, y_2 - \mathbf{k_p\, y_2} \\
\dot{y}_1^1 &= k_t^1\, y_1 - k_t^1\, y_1^1 - \mathbf{k_p\, y_1^1} \\
&\vdots \\
\dot{y}_1^{L^1-1} &= k_t^1\, y_1^{L^1-2} - k_t^1\, y_1^{L^1-1} - \mathbf{k_p\, y_1^{L^1-1}} \\
\dot{y}_2^1 &= k_t^2\, y_2 - k_t^2\, y_2^1 - \mathbf{k_p\, y_2^1} \\
&\vdots \\
\dot{y}_2^{L^2-1} &= k_t^2\, y_2^{L^2-2} - k_t^2\, y_2^{L^2-1} - \mathbf{k_p\, y_2^{L^2-1}} \\
\dot{\mathbf{q}} &= k_t^1\, y_1^{L^1-1} - k_m\, q \\
\dot{w} &= k_+^{'}\, r\,(q - w) - k_-^{'}\, w - k_w\, w - \mathbf{k_m\, w} - \mathbf{k_r\, w} \\
\dot{r} &= -k_+^{'}\, r\,(q - w) + k_-^{'}\, w + k_t^{'}\, x^{h-1} + \mathbf{k_m\, w} - \mathbf{k_r\, r} + \mathbf{k_t^2\, y_2^{L^2-1}} \\
\dot{x} &= k_w\, w - k_t^{'}\, x - \mathbf{k_r\, r} \\
\dot{x}^1 &= k_t^{'}\, x - k_t^{'}\, x^1 - \mathbf{k_r\, r} \\
&\vdots \\
\dot{x}^{H-1} &= k_t^{'}\, x^{H-2} - k_t^{'}\, x^{H-1} - \mathbf{k_r\, r}
\end{aligned}
\tag{5.2.1}
$$

Where:

$$
\begin{aligned}
q &= m + w \\
\dot{m} &= -k_+^{'}\, r\, m + k_-^{'}\, w + k_w\, w + k_t\, y^{l-1} - \mathbf{k_m\, m} + \mathbf{k_r\, w}
\end{aligned}
$$

where p, d_1, c_1, y_1 , y_1^i, d_2, c_2, y_2 , y_2^i, m, r and w are the concentrations of P, D, PD, Y, Y^i, D', PD', Y', Y'^i, RNA, R and $RRNA$ respectively.

Values of parameters for the transcription and the translation of RNA polymerase are similar to the values of parameters given before in chapter 4; for values of parameters for the ribosomal RNA , see in table (5.1):

With these values, the full system can be approximated by a reduced system, based on the same ideas of slow-fast time scales as above.

The new variables: z (total polymerase) , q (total mRNA for polymerase) and g (total ribosomes), represent the slow variables:

TABLE 5.1: Values of parameters (transcription of ribosomal RNA)

Parameter	values	**Unit**
k_+	60	$\mu\text{M}^{-1}min^{-1}$
k_-	40	min^{-1}
k_c	110	min^{-1}
k_t	5100	$\text{nucleotides.min}^{-1}$
L	6500	nucleotides
k_r	0.012	min^{-1}
d_{02}	0.0606	μM
z_0	0.5	μM

$$
\begin{aligned}
z &= \sum_{i=1}^{2} (c_i + y_i + y_i^1 + \ldots + y_i^{L^i-1}) + p \\
&\Rightarrow \dot{z} = k_t^{'} x^{h-1} - k_p z \\
g &= w + r + x + x^1 + \ldots + x^{h-1} \\
&\Rightarrow \dot{g} = -k_r g + k_t^2 y_2^{L^2-1} \\
q &= m + w \\
&\Rightarrow \dot{q} = k_t^1 y_1^{L^1-1} - k_m q
\end{aligned}
$$

5.3 Applying Tikhonov's Theorem

The fast subsystems represent the closed systems $(\sum_{i=1}^{2} (\dot{c}_i + \dot{y}_i + \dot{y}_i^1 + \ldots + \dot{y}_i^{L^i-1}) + \dot{p} = 0, \dot{w} + \dot{r} + \dot{x} + \dot{x}^1 + \ldots + \dot{x}^{h-1} = 0)$. The study of the equilibrium and the stability of the fast subsystems will not be given here, it is exactly the same as before. We get the following equations for the steady state:

$$
\begin{aligned}
y_1^{L^1-1} &= \ldots = y_1^1 = y_1 = \frac{k_c^1}{k_t^1}\, c_1 \\
y_1^{L^2-1} &= \ldots = y_2^1 = y_2 = \frac{k_c^2}{k_t^2}\, c_2 \\
c_1 &= d_0^1 \frac{p}{p + k_{11}}, \\
c_2 &= d_0^2 \frac{p}{p + k_{12}}, \\
k_{11} &= \frac{k_-^1 + k_c^1}{k_+^1} \\
k_{12} &= \frac{k_-^2 + k_c^2}{k_+^2} \\
x^{h-1} &= \ldots = x^1 = x = \frac{k_w}{k_t^{'}}\, w \\
w &= \frac{q\, r}{r + k_2} \\
k_2 &= \frac{k_-^{'} + k_w}{k_+^{'}}
\end{aligned}
\tag{5.3.1}
$$

The slow subsystem is given by:

$$
\begin{aligned}
\dot{z} &= k_t^{'}\, x^{h-1} - k_p\, z \\
\dot{q} &= k_t^1\, y_1^{L^1-1} - k_m\, q \\
\dot{g} &= k_t^2\, y_2^{L^2-1} - k_r\, g
\end{aligned}
\tag{5.3.2}
$$

5.4 The reduced system

Replacing the fast variables $(x^{h-1}, y_1^{L^1-1}, y_2^{L^2-1})$ by the equations of the equilibrium of the fast subsystem, we obtain this reduced system:

$$
\begin{aligned}
\dot{z} &= k_w \frac{q\, r(q,g)}{r(q,g) + k_2} - k_p\, z \\
\dot{q} &= k_c^1 d_0^1 \frac{p(z)}{p(z) + k_{11}} - k_m\, q \\
\dot{g} &= -k_r\, g + k_c^2 d_0^2 \frac{p(z)}{p(z) + k_{12}}
\end{aligned}
\tag{5.4.1}
$$

where $p(z)$ and, $r(q,g)$ can be calculated from these algebraic equations (as above, the solution is unique):

$$
\begin{aligned}
&p + d_0^1 \frac{p}{p + k_{11}} + L^1 \frac{k_c^1}{k_t^1} d_0^1 \frac{p}{p + k_{11}} + d_0^2 \frac{p}{p + k_{12}} + L^2 \frac{k_c^2}{k_t^2} d_0^2 \frac{p}{p + k_{12}} = z \\
&r + \frac{q\, r}{r + k_2} + H \frac{k_w}{k_t'} \frac{q\, r}{r + k_2} = g
\end{aligned}
\tag{5.4.2}
$$

Consider that:

$$
\begin{aligned}
\gamma_1 &= (L^1 \frac{k_c^1}{k_t^1} + 1)\, d_0^1 \\
\gamma_2 &= (L^2 \frac{k_c^2}{k_t^2} + 1)\, d_0^2 \\
\lambda &= H \frac{k_w}{k_t'} + 1.
\end{aligned}
\tag{5.4.3}
$$

The algebraic equations becomes:

$$
\begin{aligned}
z &= \gamma_1 \frac{p(z)}{p(z) + k_{11}} + \gamma_2 \frac{p(z)}{p(z) + k_{12}} + p(z) \\
g &= \lambda \frac{q\, r(q,g)}{r(q,g) + k_2} + r(q,g).
\end{aligned}
\tag{5.4.4}
$$

If we differentiate the first equation with respect to z, the second equation with respect to g and q, we obtain:

$$
\begin{aligned}
1 &= \gamma_1 \frac{k_{11}}{(p(z) + k_{11})^2} \frac{\partial p(z)}{\partial z} + \gamma_2 \frac{k_{12}}{(p(z) + k_{12})^2} \frac{\partial p(z)}{\partial z} + \frac{\partial p(z)}{\partial z} \\
1 &= \lambda q \frac{k_2}{(r(q,g) + k_2)^2} \frac{\partial r(q,g)}{\partial g} + \frac{\partial r(q,g)}{\partial g} \\
0 &= \lambda \frac{r(q,g)}{r(q,g) + k_2} + \lambda q \frac{k_2}{(r(q,g) + k_2)^2} \frac{\partial r(q,g)}{\partial q} + \frac{\partial r(q,g)}{\partial q}.
\end{aligned}
\tag{5.4.5}
$$

If we differentiate again equations 5.4.5, the first equation with respect to z, and the second equation with respect to g and the third equation with respect to q and after with respect to g, we obtain:

$$\begin{aligned}
\frac{\partial^2 p(z)}{\partial z^2} &= \gamma_1 \frac{2k_{11}}{(p(z)+k_{11}))^3}\frac{\partial p(z)}{\partial z}^2 + \gamma_2 \frac{2k_{12}}{(p(z)+k_{12}))^3}\frac{\partial p(z)}{\partial z}^2 \\
\frac{\partial^2 r(q,g)}{\partial g^2} &= \lambda q \frac{2k_2}{(r(q,g)+k_2)^3}\frac{\partial r(q,g)}{\partial g}^2 \\
\frac{\partial^2 r(q)}{\partial q^2} &= \frac{-2\lambda \frac{k_2}{(r(q)+k_2)^2}\frac{\partial r(q)}{\partial q} + \lambda q \frac{2k_2}{(r(q)+k_2)^3}\frac{\partial r(q)}{\partial q}^2}{1+\lambda q \frac{k_2}{(r(q)+k_2)^2}} \\
\frac{\partial^2 r(q,g)}{\partial g \partial q} &= -\lambda \frac{k_2}{(r(q,g)+k_2)^2}\frac{\partial r(q,g)}{\partial g} + \lambda q \frac{2k_2}{(r(q,g)+k_2)^3}\frac{\partial r(q,g)}{\partial g}\frac{\partial r(q,g)}{\partial q}.
\end{aligned} \tag{5.4.6}$$

Which means that:

- $p(z)$ is positive, increasing and convex with respect to z.
- $r(q,g)$ is positive, increasing and convex with respect to g.
- $r(q,g)$ is positive, decreasing, and convex with respect to q
- $\frac{\partial^2 r(q,g)}{\partial g \partial q}$ is decreasing because $\frac{\partial r(q,g)}{\partial g}$ is positive and $\frac{\partial r(q,g)}{\partial q}$ is negative.

These properties will be useful in the next sections.

5.5 Dynamical study of the reduced system

The Jacobian matrix of the reduced system 5.4.1 is:

$$J(z,q,g) = \begin{pmatrix} -k_p & k_w \frac{\partial}{\partial q}\left(\frac{q\, r(q,g)}{r(q,g)+k_2}\right) & k_w \frac{\partial}{\partial g}\left(\frac{q\, r(q,g)}{r(q,g)+k_2}\right) \\ k_c^1 d_0^1 \frac{\partial}{\partial z}\left(\frac{p(z)}{p(z)+k_{11}}\right) & -k_m & 0 \\ k_c^2 d_0^2 \frac{\partial}{\partial z}\left(\frac{p(z)}{p(z)+k_{12}}\right) & 0 & -k_r \end{pmatrix} \tag{5.5.1}$$

We will see below that the off-diagonal elements of the Jacobian matrix are nonnegative, therefore the reduced system 5.4.1 is monotone, but we have for example:

$$\frac{\partial^2}{\partial g \partial q}\left(\frac{q\, r(q,g)}{r(q,g)+k_2}\right) = \frac{\partial^2}{\partial g \partial q}(g - r(q,g)) = -\frac{\partial^2}{\partial g \partial q} r(q,g)$$

which is an increasing function, because $\frac{\partial^2 r(q,g)}{\partial g \partial q}$ is a decreasing function with respect to q et g. The Jacobian matrix is not decreasing and we can not apply as previously in chapter 4 the theorem of monotone system with a decreasing Jacobian matrix, see appendix A.4. Therefore we have to compute explicitly the equilibria of the system.

5.5.1 Equilibria of the reduced system

We have at the equilibrium:

$$\begin{aligned} z &= \mu_1 \frac{q\, r(q,g)}{r(q,g)+k_2} \\ q &= \mu_2 \frac{p(z)}{p(z)+k_{11}} \\ g &= \mu_3 \frac{p(z)}{p(z)+k_{12}} \\ z &= \gamma_1 \frac{p(z)}{p(z)+k_{11}} + \gamma_2 \frac{p(z)}{p(z)+k_{12}} + p(z) \\ g &= \lambda \frac{q\, r(q,g)}{r(q,g)+k_2} + r(q,g). \end{aligned} \tag{5.5.2}$$

with: $\mu_1 = \frac{k_w}{k_p}$, $\mu_2 = \frac{k_c^1 d_0^1}{k_m}$ and $\mu_3 = \frac{k_c^2 d_0^2}{k_r}$.

which imply:

$$\begin{aligned} \mu_1 \frac{q\, r(q,g)}{r(q,g)+k_2} &= \gamma_1 \frac{p(z)}{p(z)+k_{11}} + \gamma_2 \frac{p(z)}{p(z)+k_{12}} + p(z) \\ \mu_3 \frac{p(z)}{p(z)+k_{12}} &= \lambda \frac{q\, r(q,g)}{r(q,g)+k_2} + r(q,g) \\ q &= \mu_2 \frac{p(z)}{p(z)+k_{11}}. \end{aligned} \tag{5.5.3}$$

First, we notice that the origin $(0,0,0)$ is an equilibrium. When the equilibrium is different from $(0,0,0)$, from the first equation in (5.5.3), we get:

$$\frac{r(p)}{r(p)+k_2} = \frac{1}{\mu_1\mu_2}(\gamma_1 + \gamma_2 \frac{p+k_{11}}{p+k_{12}} + p + k_{11}) \tag{5.5.4}$$

let

$$\varphi_1(p) = \frac{1}{\mu_1\mu_2}(\gamma_1 + \gamma_2 \frac{p+k_{11}}{p+k_{12}} + p + k_{11}).$$

Therefore:

$$\begin{aligned} r(p) &= \frac{k_2\varphi_1(p)}{1-\varphi_1(p)} \\ &= \frac{-k_2(p^2 + (\gamma_1+\gamma_2+k_{12}+k_{11})p + \gamma_1 k_{12} + \gamma_2 k_{11} + k_{11}k_{12})}{p^2 + (\gamma_1+\gamma_2+k_{12}+k_{11}-\mu_1\mu_2)p - \mu_1\mu_2 k_{12} + \gamma_1 k_{12} + \gamma_2 k_{11} + k_{11}k_{12}} \end{aligned} \tag{5.5.5}$$

To have a positive solutions, we should have $\varphi_1(p) < 1$, which implies:

$$p^2 + (\gamma_1 + \gamma_2 + k_{12} + k_{11} - \mu_1\mu_2)p - \mu_1\mu_2k_{12} + \gamma_1k_{12} + \gamma_2k_{11} + k_{11}k_{12} < 0$$

Which implies that $p \in]p_1, p_2[$, with $p_1 = \frac{1}{2}(\mu_1\mu_2 - \gamma_1 - \gamma_2 - k_{12} - k_{11} - \sqrt{A})$, $p_2 = \frac{1}{2}(\mu_1\mu_2 - \gamma_1 - \gamma_2 - k_{12} - k_{11} + \sqrt{A})$, and $A = 2\mu_1\mu_2k_{12} - 2\gamma_1k_{12} - 2\gamma_2k_{11} - 2k_{11}k_{12} + 2k_{12}\gamma_2 + k_{12}^2 + \mu_1^2\mu_2^2 + \gamma_1^2 + 2\gamma_1\gamma_2 + 2\gamma_1k_{11} + \gamma_2^2 + k_{11}^2 - 2\mu_1\mu_2\gamma_1 - 2\mu_1\mu_2\gamma_2 - 2\mu_1\mu_2k_{11}$.

From the second equation in (5.5.3), we also get:

$$\begin{aligned} r(p) &= \mu_3\frac{p}{p+k_{12}} - \frac{\lambda}{\mu_1}(\gamma_1\frac{p}{p+k_{11}} + \gamma_2\frac{p}{p+k_{12}} + p) \\ &= (\mu_3 - \frac{\lambda}{\mu_1}\gamma_2)\frac{p}{p+k_{12}} - \frac{\lambda}{\mu_1}(\gamma_1\frac{p}{p+k_{11}} + p) \end{aligned} \tag{5.5.6}$$

$r(p)$ should be positive, therefore we have this constraint: $\mu_3 - \frac{\lambda}{\mu_1}\gamma_2 > 0$, to have other equilibria.

Consider the functions:

$$\begin{aligned} \psi_1 &= \frac{k_2\varphi_1(p)}{1-\varphi_1(p)} \\ \psi_2 &= (\mu_3 - \frac{\lambda}{\mu_1}\gamma_2)\frac{p}{p+k_{12}} - \frac{\lambda}{\mu_1}(\gamma_1\frac{p}{p+k_{11}} + p) \end{aligned} \tag{5.5.7}$$

Therefore we have to study the intersections between these two above functions ψ_1 and ψ_2 to know how many equilibria exist:

we have:

$$\begin{aligned} \psi_1' &= \frac{k_2}{(1-\varphi_1(p))^2}\varphi_1'(p) \\ &= \frac{k_2}{(1-\varphi_1(p))^2}\frac{1}{\mu_1\mu_2}(\gamma_2\frac{k_{12}-k_{11}}{p+k_{12}} + 1) \\ \psi_1'' &= \frac{k_2}{(1-\varphi_1(p))^3}\varphi_1'(p)^2 \end{aligned} \tag{5.5.8}$$

Taking our values of parameters as above, we get $k_{12} - k_{11}$ positive. Then ψ_1 is an increasing and convex function with respect to p, with $\psi_1(0) = \frac{k_2\varphi_1(0)}{1-\varphi_1(0)} = \frac{k_2(\gamma_1k_{12}+\gamma_2k_{11}+k_{11}k_{12})}{\mu_1\mu_2k_{12}-\gamma_1k_{12}-\gamma_2k_{11}-k_{11}k_{12}}$ and with the constraint $\varphi_1(p) < 1$.

We also have

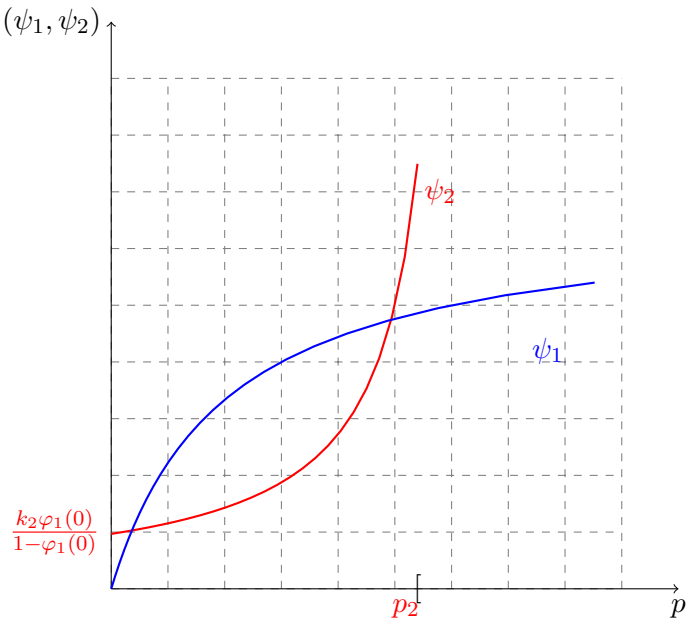

FIGURE 5.5.1: Two equilibria exist: one near the origin which corresponds to $r \approx \psi_1(0) = \frac{k_2(\gamma_1 k_{12}+\gamma_2 k_{11}+k_{11}k_{12})}{\mu_1\mu_2 k_{12}-\gamma_1 k_{12}-\gamma_2 k_{11}-k_{11}k_{12}}$ and another one far from the origin which corresponds to $p >> 0 \approx p_2$ $(\varphi_1(p_2) = 1)$ and $r >> 0$.

$$\begin{aligned}\psi_2' &= (\mu_3 - \frac{\lambda}{\mu_1}\gamma_2)\frac{k_{12}}{(p+k_{12})^2} - \frac{\lambda}{\mu_1}(\gamma_1\frac{k_{11}}{(p+k_{11})^2} + 1)\\ \psi_1'' &= -(\mu_3 - \frac{\lambda}{\mu_1}\gamma_2)2\frac{k_{12}}{(p+k_{12})^3} + 2\frac{\lambda}{\mu_1}\gamma_1\frac{k_{11}}{(p+k_{11})^3}\end{aligned} \tag{5.5.9}$$

With our values of parameters $\psi_2(p)$ is an increasing and concave function with respect to $p \in [0, p_2]$.

The drawing of functions $\psi_1(p)$ and $\psi_2(p)$ is shown in figure 5.5.1. Therefore two equilibria exist: one close to the origin and another one far from the origin.

We solve numerically the equation $\psi_1(p) = \psi_2(p)$ with our values of parameters above and for p positive, therefore it gives three solutions which are: $p = 0.969\ e^{-4}, p = 198.846$, and $p = 1.8103e^5$.

- For $p = 0.969\ \ e^{-4}$, we obtain: $r(p) := 0.1845$, $q = \frac{\mu_2 p}{(p+k_{11})} = 0.324\ e^{-5}$., $z = \frac{\mu_1 qr}{(r+k_2)} = 0.00024$, $g = \frac{\mu_3 p}{(p+k12)} = 0.1845$.
- For $p = 198.846$, $r(p) := 4696.965$, $q = 0.3048\ e^{-1}$, $z = 202.475$, $g = 4702.31$.
- For $p = 1.8103\ e^5$ we have $r(p) = -16.38$ which is negative, so this solution does not respect the constraint $(r(p) > 0)$, and is neglected.

Therefore, for the reduced system we have three equilibria which are: $(0,0,0)$, $(0.00024,$ $0.324\ e^{-5}, 0.1845)$, $(202.47, 0.3048\ e^{-1}, 4702.31)$.

5.5.2 Stability of the equilibria

5.5.2.1 Stability of the origin

To study the local stability at the origin, we have to compute the Jacobian matrix near $(z^*, q^*, g^*) = (0,0,0)$:

$$J(z,q,g) = \begin{pmatrix} -k_p & -\frac{k_w}{\lambda}\frac{\partial r(q,g)}{\partial q} & \frac{k_w}{\lambda}(1-\frac{\partial r(q,g)}{\partial g}) \\ k_c^1 d_0^1 \frac{k_{11}}{(p(z)+k_{11})^2}\frac{\partial p(z)}{\partial z} & -k_m & 0 \\ k_c^2 d_0^2 \frac{k_{12}}{(p(z)+k_{12})^2}\frac{\partial p(z)}{\partial z} & 0 & -k_r \end{pmatrix} \tag{5.5.10}$$

We have:

$$\begin{aligned} \frac{\partial p(z)}{\partial z} &= \frac{1}{\gamma_1 \frac{k_{11}}{(p(z)+k_{11})^2} + \gamma_2 \frac{k_{12}}{(p(z)+k_{12})^2} + 1} \\ \frac{\partial r(q,g)}{\partial g} &= \frac{1}{\lambda q \frac{k_2}{(r(q,g)+k_2)^2} + 1} \\ \frac{\partial r(q,g)}{\partial q} &= -\frac{\lambda \frac{r(q,g)}{r(q,g)+k_2}}{\lambda q \frac{k_2}{(r(q,g)+k_2)^2} + 1}. \end{aligned} \tag{5.5.11}$$

We have, when $z = 0$, then $p(z) = 0$. Similarly, if $(q,g) = (0,0)$, then $r(q,g) = 0$, therefore:

$$\begin{aligned} \frac{k_{11}}{(p(z)+k_{11})^2}\frac{\partial p(z)}{\partial z}\bigg|_{(0,0,0)} &= \frac{k_{12}}{(\gamma_1+k_{11})k_{12}+\gamma_2 k_{11}} \\ \frac{k_{12}}{(p(z)+k_{12})^2}\frac{\partial p(z)}{\partial z}\bigg|_{(0,0,0)} &= \frac{k_{11}}{(\gamma_2+k_{12})k_{11}+\gamma_1 k_{12}} \\ \frac{\partial r(q,g)}{\partial g}\bigg|_{(0,0,0)} &= 1 \\ \frac{\partial r(q,g)}{\partial q}\bigg|_{(0,0,0)} &= 0. \end{aligned} \tag{5.5.12}$$

Therefore, the Jacobian matrix at the origin $(0,0,0)$ is:

$$J(z,q,g)|_{(0,0,0)} = \begin{pmatrix} -k_p & 0 & 0 \\ \frac{k_c^1 d_0^1 k_{12}}{(\gamma_1+k_{11})k_{12}+\gamma_2 k_{11}} & -k_m & 0 \\ \frac{k_c^2 d_0^2 k_{11}}{(\gamma_2+k_{12})k_{11}+\gamma_1 k_{12}} & 0 & -k_r \end{pmatrix} \tag{5.5.13}$$

The Jacobian matrix at the origin $(0,0,0)$ is a triangular matrix with a negative diagonal, so the eigenvalues are real negative, therefore $(0,0,0)$ is locally stable.

5.5.2.2 Stability of the second equilibrium which is near to the origin

This equilibrium $(z^*, q^*, g^*) = (0.00024, 0.324\ e^{-5}, 0.184)$, corresponds to the case that $p(z) << k_{11}$, $p(z) << k_{12}$, $r(q,g) << k_2$, therefore if we apply these approximations, the reduced system 5.4.1 becomes:

$$\begin{aligned} \dot{z} &= \frac{k_w}{k_2} q\, r(q,g) - k_p\, z \\ \dot{q} &= \frac{k_c^1 d_0^1}{k_{11}} p(z) - k_m\, q \\ \dot{g} &= \frac{k_c^2 d_0^2}{k_{12}} p(z) - k_r\, g \end{aligned} \tag{5.5.14}$$

with these algebraic equations:

$$\begin{aligned} z &= \gamma_1 \frac{p(z)}{k_{11}} + \gamma_2 \frac{p(z)}{k_{12}} + p(z) \\ g &= \lambda \frac{q\, r(q,g)}{k_2} + r(q,g). \end{aligned} \tag{5.5.15}$$

where we easily obtain the following expressions of $p(z)$ as a function of z and $r(q,g)$ as a function of q and g:

$$\begin{aligned} p(z) &= \frac{z}{\frac{\gamma_1}{k_{11}} + \frac{\gamma_2}{k_{12}} + 1} \\ r(q,g) &= \frac{g}{\frac{\lambda}{k_2} q + 1} \end{aligned} \tag{5.5.16}$$

Therefore, by replacing $p(z)$ and $r(q,g)$ in the 5.5.14, we get the following reduced system:

$$\begin{aligned}
\dot{z} &= k_w \frac{q}{k_2 + \lambda q} g - k_p\, z \\
\dot{q} &= \frac{k_c^1 d_0^1}{k_{11}} \frac{z}{\frac{\gamma_1}{k_{11}} + \frac{\gamma_2}{k_{12}} + 1} - k_m\, q \\
\dot{g} &= \frac{k_c^2 d_0^2}{k_{12}} \frac{z}{\frac{\gamma_1}{k_{11}} + \frac{\gamma_2}{k_{12}} + 1} - k_r\, g
\end{aligned} \tag{5.5.17}$$

Therefore, for the equilibrium we obtain:

$$\begin{aligned}
k_w \frac{q^*}{k_2 + \lambda q^*} g^* - k_p\, z^* &= 0 \\
q^* &= \frac{\mu_2}{k_{11}} \frac{z^*}{\frac{\gamma_1}{k_{11}} + \frac{\gamma_2}{k_{12}} + 1} \\
g^* &= \frac{\mu_3}{k_{12}} \frac{z^*}{\frac{\gamma_1}{k_{11}} + \frac{\gamma_2}{k_{12}} + 1}
\end{aligned} \tag{5.5.18}$$

with $\mu_2 = \frac{k_c^1 d_0^1}{k_m}$ and $\mu_3 = \frac{k_c^2 d_0^2}{k_r}$. Therefore we get:

$$\left(\frac{\mu_2 \mu_3 k_{12} k_{11} k w z^*}{(k_2(\gamma_1 k_{12} + \gamma_2 k_{11} + k_{11} k_{12}) + \lambda \mu_2 z^* k_{12})(\gamma_1 k_{12} + \gamma_2 k_{11} + k_{11} k_{12})} - k_p\right) z^* = 0$$

Then, we obtain, either:

$$\begin{aligned}
z^* \quad &= 0, \\
q^* \quad &= 0, g^* = 0.
\end{aligned} \tag{5.5.19}$$

or

$$z^* = \frac{k_p k_2 (\gamma_1 k_{12} + \gamma_2 k_{11} + k_{11} k_{12})^2}{\mu_2 \mu_3 k w k_{12} k_{11} - k_p \lambda \mu_2 k_{12} (\gamma_1 k_{12} + \gamma_2 k_{11} + k_{11} k_{12})}$$

and,

$$\begin{aligned}
q^* &= \frac{k_p k_2 (\gamma_1 k_{12} + \gamma_2 k_{11} + k_{11} k_{12})}{k w k_{11} \mu_3 - k_{12} k_p \lambda \gamma_1 - k_{12} k_p \lambda k_{11} - k_p \lambda \gamma_2 k_{11}} \\
g^* &= \frac{k_{11} k_2 k_p \mu_3 (\gamma_1 k12 + \gamma_2 k_{11} + k_{11} k_{12})}{k_{12} \mu_2 (k_w k_{11} \mu_3 - k_{12} k_p \lambda \gamma_1 - k_{12} k_p \lambda k_{11} - k_p \lambda \gamma_2 k_{11})}
\end{aligned}$$

Therefore for the same value of parameters as above we obtain the following equilibrium near to the origin:

$$\begin{aligned}
z^* &= 0.00024, \\
q^* &= 0.0000032, g^* \quad = 0.1825.
\end{aligned} \tag{5.5.20}$$

The Jacobian matrix of the reduced system 5.5.17 is

$$\begin{pmatrix} -k_p & k_w \frac{k_2}{(k_2+\lambda q)^2} g & k_w \frac{q}{k_2+\lambda q} \\ C_1 & -k_m & 0 \\ C_2 & 0 & -k_r \end{pmatrix} \tag{5.5.21}$$

With: $C_1 = \frac{k_c^1 d_0^1}{k_{11}(\frac{\gamma_1}{k_{11}}+\frac{\gamma_2}{k_{12}}+1)}$ and $C_2 = \frac{k_c^2 d_0^2}{k_{12}(\frac{\gamma_1}{k_{11}}+\frac{\gamma_2}{k_{12}}+1)}$.

So, the characteristic polynomial of the Jacobian matrix near the equilibrium $J(z^*, q^*, g^*)$ is given by:

$$\lambda^3 + (k_m + k_p + k_r)\lambda^2 + ((k_m + k_p)k_r + k_p k_m - k_w C_1 \frac{k_2}{(k_2 + \lambda q^*)^2} g^* k_w C_2 \frac{q^*}{k_2 + \lambda q^*})\lambda$$
$$+ k_r k_p k_m - k_w C_1 \frac{k_2}{(k_2 + \lambda q^*)^2} g^* k_r - k_w C_2 \frac{q^*}{k_2 + \lambda q^*} k_m = 0$$

Taking the same value of parameter as above the term:

$$k_r k_p k_m - k_w C_1 \frac{k_2}{(k_2 + \lambda q^*)^2} g^* k_r - k_w C_2 \frac{q^*}{k_2 + \lambda q^*} k_m = -det(J(z^*, q^*, g^*)) = -0.002053$$

is negative, this means that Routh criterion is not satisfied, and the equilibrium (0.00024;0.0000032; 0.1825) which is near the origin is locally unstable.

5.5.2.3 Stability of the third equilibrium which is far from the origin

For the case that the equilibrium $(z^*, q^*, g^*) = (202.47, 0.3048\ e^{-1}, 4702.31)$, $(p = 198.85, r = 4696.96)$, we have $p(z) >> k_{11}$, $p(z) >> k_{12}$, $r(q, g) >> k_2$, therefore if we apply these approximations, the reduced system 5.4.1 becomes:

$$\begin{aligned} \dot{z} &= k_w q - k_p z \\ \dot{q} &= k_c^1 d_0^1 - k_m q \\ \dot{g} &= k_c^2 d_0^2 - k_r g \end{aligned} \tag{5.5.22}$$

Therefore, for the equilibrium we obtain:

$$\begin{aligned} z^* &= \frac{k_w k_c^1 d_0^1}{k_p k_m} \\ q^* &= \frac{k_c^1 d_0^1}{k_m} \\ g^* &= \frac{k_c^2 d_0^2}{k_r}. \end{aligned} \tag{5.5.23}$$

Taking the value of parameter as above:

$$\begin{aligned} z^* &= 204.12 \\ q^* &= 0.0306, g^* = 4761.43. \end{aligned} \tag{5.5.24}$$

The Jacobian matrix of the reduced systems 5.5.22 is

$$\begin{pmatrix} -k_p & k_w & 0 \\ 0 & -k_m & 0 \\ 0 & 0 & -k_r \end{pmatrix} \tag{5.5.25}$$

Therefore, the equilibrium (204.12;0.0306; 4761.43) is locally stable.

5.5.3 The global stability of the equilibria

The Jacobian matrix is given above, see 5.5.10. We have:

- $\frac{\partial}{\partial q}(\frac{q\, r(q,g)}{r(q,g)+k_2}) = \frac{\partial}{\partial q}(g - r(q,g)) = -\frac{\partial}{\partial q} r(q,g)$, positive, because $r(q,g)$ is decreasing with q.
- $\frac{\partial}{\partial g}(\frac{q\, r(q,g)}{r(q,g)+k_2}) = q\frac{k_2}{(r(q,g)+k_2)^2}\frac{\partial}{\partial g}(r(q,g))$, positive, because $\frac{\partial r(q,g)}{\partial g}$ is positive.
- $\frac{\partial}{\partial z}(\frac{p(z)}{p(z)+k_{11}}) = \frac{k_{11}}{(p(z)+k_{11})^2}\frac{\partial p(z)}{\partial z}$, which is positive, because $\frac{\partial p(z)}{\partial z}$ is positive.
- Similarly, for $\frac{\partial}{\partial z}(\frac{p(z)}{p(z)+k_{12}}) = \frac{k_{12}}{(p(z)+k_{12})^2}\frac{\partial p(z)}{\partial z}$, which is positive.

Therefore the reduced system is monotone.

We have also:

- $\frac{p(z)}{p(z)+k_{11}}$ is bounded, therefore for q large $\dot{q} = k_c^1 d_0^1 \frac{p(z)}{p(z)+k_{11}} - k_m\, q$ is negative.
- similarly, $\dot{g} = -k_r\, g + k_c^2 d_0^2 \frac{p(z)}{p(z)+k_{12}}$ is non-positive for g large.

- $\frac{q\, r(q,g)}{r(q,g)+k_2}$ is also bounded because g is bounded ($g = \lambda \frac{q\, r(q,g)}{r(q,g)+k_2} + r(q,g)$), so for z large $\dot{z} = k_w \frac{q\, r(q,g)}{r(q,g)+k_2} - k_p\, z$ is negative.

  Which means that z, q, and g are bounded.

We obtain that we have three equilibrium, one stable at the origin, an other one near to the origin (unstable) and an other one far from the origin (stable). The equilibrium which is near to the origin is an unstable saddle point, see [57]. The reduced system 5.4.1 is monotone and the solutions are bounded, therefore as before we can apply the same techniques from monotone systems, to obtain that the origin is globally stable in the rectangle limited by $(0,0,0)$ and a point near and smaller than the second equilibrium (unstable), and the third equilibrium (positive and stable) is globally stable in the positive orthant starting from a point near and greater than the second equilibrium (unstable).

5.6 Conclusions

We studied in this chapter a general model of the genetic machinery, taking the previous model in chapter 4 for the polymerase and coupling it with a model for the synthesis of ribosomes. We proved that the system have three equilibria; the first one in the origin which is stable, another one positive and near to the origin which is unstable and the last one far from the origin which is stable. We briefly used the same techniques from monotone system theory to study the global stability. An interesting generalization would be to consider the input function ppGpp (which inhibits transcription of the ribosomes) to obtain the commutations between two cases which depends on the concentration of ppGpp: either the model in this chapter or the model in the previous chapter.

Chapter 6

Control of small genetic networks systems

The oscillator made of a negative loop of two genes is one of the most classical motifs of genetic networks. We give solutions to control such an oscillator by modifying the synthesis rates. Our models are given by Piecewise Affine systems, and the control is very qualitative, taking only two values. The necessary measurements for implementing this control only depend on the fact that some gene is expressed or not. Our goal is to obtain sustained oscillations. Then we study the control by a sliding mode of the oscillator given by an ODE, to suppress the oscillations.

6.1 Introduction

Due to the huge quantity of high-throughput experimental data, modelling of genetic networks is now a useful and compulsory tool for the study of biological genetic networks. Several formalisms exist (see the review [17]), from the very qualitative Boolean modelling ([64]) to the exhaustive mass action chemical type modelling of each of the elementary steps of the transcription translation processes [42]. In this chapter, to build dynamical models, we will use classical ordinary differential equations or, most frequently, the (equally classical) Piecewise Affine (PWA) formalism made with discontinuous differential equations.

Due to the enormous progress in genetic manipulations, it becomes now possible to act upon the expression of one gene at the transcriptional or translational level. For example, biochemical inducers may interfere with the main gene transcription effectors, and, depending on the quantity of inducer injected in the medium, the expression of

some gene may be slowed down. On the basis of these recent progresses in experimental synthetic biology [22, 27, 65], we assume that synthesis rates can be controlled by the biologist.

The use of control in mathematical modelling of genetic networks is less classical because the experimental means are quite recent. Moreover, the classical mathematical tools of Control Theory [59], a branch of Applied Mathematics, are rarely applicable. The classical theory is exhaustively developed for linear systems, with control of any sign (positive or negative). On the contrary, the controlled systems obtained from genetic controlled networks are very often non-linear, and the control has often a sign, because of the positive nature of biochemical concentrations for example, or because it is possible to add an inducer in the medium but not to take it away.

Moreover, the control for gene expression is experimentally often very qualitative [60]: the concentrations of proteins or the expression of genes are only qualitatively known (some gene may be known to be on or off), and the control itself has often only a small number of value. It is not possible to exert a control with a good precision, depending of a variable measured with a good precision, as done in classical Control Theory.

This chapter wants to bring some lights on possible solutions to these difficult problems in a particular case. Some formalisms and works already exist with qualitative control. In the paper [13], the authors have controlled in a very qualitative way one the most famous motif of two-gene system: the bistable switch, a positive loop of two genes inhibiting each other. The dynamics of this system is typically a two states system, with two stable attractors (and an unstable one between the two), and the system may switch between the two (as indicated by the name). In this chapter, we will use slightly different techniques, to control the other classical motif of two genes: the oscillator, a system build by a negative loop of two genes, that can generate sustained or damped oscillations. Our aim for control will be to obtain a real periodic behaviour, when the behaviour without control is damped oscillations converging toward an equilibrium. We consider PWA differential system, and a qualitative control that only changes from domain to domain, and therefore is a constant within a rectangular domain. The resulting controlled system is therefore still PWA systems, and we use known tools existing for these systems to obtain our results. The control is multiplicative with respect to the synthesis rate, and must be positive. In [21], the authors address a similar problem with affine additive control and a unique threshold per variables.

Our work has some relations with theoretical qualitative control techniques used for piecewise linear systems in the field of genetic regulatory networks ([13]). The approach is also similar to the domain approaches used in hybrid systems theory, where there are some (controlled) transitions between regions, forming a transition graph [7, 35].

6.2 The genetic oscillator

As explained above, one main motif in biological feedback loops is the negative loop, which is, under its simplest form, a negative loop with two genes: the first gene activates the production of the protein of the second gene, which inhibits the synthesis of the first protein (Fig. 1.5.3). The classical model for this negative loop is:

$$\begin{cases} \dot{x_1} = & k_1\, h^-(x_2, \theta_2, n) - \gamma_1 x_1 \\ \dot{x_2} = & k_2\, h^+(x_1, \theta_1, n) - \gamma_2 x_2 \end{cases} \tag{6.2.1}$$

where h^+ and h^- are the increasing or decreasing Hill functions:

$h^+(x, \theta, n) = x^n/(x^n + \theta^n),\ h^-(x, \theta, n) = \theta^n/(x^n + \theta^n)$

The dynamical behaviour of this system is rather easy to study; if we suppose that the following inequalities are fulfilled

$$\theta_1 < \frac{k_1}{\gamma_1};\ \ \theta_2 < \frac{k_2}{\gamma_2}$$

then the system has oscillations. A classical study gives that there is a unique equilibrium, which is shown to be locally and globally stable (local stability is very easy because the trace of the Jacobian matrix is negative and the determinant is positive). Therefore the observed behaviour is damped oscillations converging to the equilibrium, simulations are given Fig. 1.5.5.

We are now interested in a *qualitative* description of this oscillator, corresponding to the case $n_i \to \infty$ where the sigmoidal functions h^+ and h^- become step functions s. Without loss of generality, we will consider only the case when the system is defined inside the (invariant) set $[0, k_1/\gamma_1] \times [0, k_2/\gamma_2]$:

$$\begin{aligned} \dot{x}_1 &= k_1 s^-(x_2, \theta_2) - \gamma_1 x_1, \\ \dot{x}_2 &= k_2 s^+(x_1, \theta_1) - \gamma_2 x_2. \end{aligned} \tag{6.2.2}$$

This class of piecewise affine systems (PWA) was first introduced by L. Glass [29], and is widely used for modeling genetic regulatory networks [12, 14, 18, 29]. Step functions are not defined at threshold points, but solutions of the differential system on a threshold can still be defined in the sense of Filippov, as the solutions of differential inclusions.

The dynamics of system 6.2.2 can be divided into four regions, or *domains*, where the vector field is defined and very simple (linear):

$$\begin{aligned} B_{00} &= \{x \in \mathbb{R}^2_{\geq 0} : \ 0 < x_1 < \theta_1, \ 0 < x_2 < \theta_2\} \\ B_{01} &= \{x \in \mathbb{R}^2_{\geq 0} : \ 0 < x_1 < \theta_1, \ \theta_2 < x_2 < \kappa_2/\gamma_2\} \\ B_{10} &= \{x \in \mathbb{R}^2_{\geq 0} : \ \theta_1 < x_1 < \kappa_1/\gamma_1, \ 0 < x_2 < \theta_2\} \\ B_{11} &= \{x \in \mathbb{R}^2_{\geq 0} : \ \theta_1 < x_1 < \kappa_1/\gamma_1, \ \theta_2 < x_2 < \kappa_2/\gamma_2\}. \end{aligned}$$

In addition, there are also *switching domains*, where the system is defined only as a differential inclusion, corresponding to the segments where each of the variables is at a threshold ($x_i = \theta_i$ and $x_j \in [0, k_j/\gamma_j]$). We do not need to use this approach here, because the vector fields from the two domains around the threshold are transverse to the segment, and therefore the solution of the differential equation is well defined.

In each of the regular domains, the analytic solution of decoupled linear (affine) equations can be obtained. To simplify the algebraic expressions that could become very long, we make the assumptions that the degradation rates are equal (that implies that trajectories are in fact straight lines, and simplify the computations). Our methodology and the results would remain the same with or without this assumption.

Assumption 1. From now on, $\gamma_1 = \gamma_2 = \gamma$.

The study of such an oscillator has already been done [31], we recall some results that will be useful for control. We have now four domains and four differential systems:

- in B_{00} $\begin{cases} \dot{x_1} = & k_1 - \gamma x_1 \\ \dot{x_2} = & -\gamma x_2 \end{cases}$
- in B_{10} $\begin{cases} \dot{x_1} = & k_1 - \gamma x_1 \\ \dot{x_2} = & k_2 - \gamma x_2 \end{cases}$
- in B_{11} $\begin{cases} \dot{x_1} = & -\gamma x_1 \\ \dot{x_2} = & k_2 - \gamma x_2 \end{cases}$
- in B_{01} $\begin{cases} \dot{x_1} = & -\gamma x_1 \\ \dot{x_2} = & -\gamma x_2 \end{cases}$

Within each domain, the solution converge toward a focal point ϕ_{ij} outside of the domain; therefore the solution will cross the threshold and continue in the next domain. The possible transition between domains are given by the transition graph (see [12] for explanations).

As in the continuous case, we add some constraints on the (positive) parameters to obtain an oscillatory behaviour:

Assumption 2. $\theta_1 < \frac{k_1}{\gamma}$ and $\theta_2 < \frac{k_2}{\gamma}$

Numerical simulation is given Fig. 1.6.3.

We make the change of variables $t' = \gamma t$, $y_1 = x_1 - \theta_1, y_2 = x_2 - \theta_2$ and define the first-return map starting from the boundary $y_1 = y_1^0 < 0, y_2 = 0$. Defining $\alpha = \frac{k_1}{\gamma} - \theta_1$, $\beta = \frac{k_2}{\gamma} - \theta_2$,$\delta = (\theta_1\beta + \alpha\beta + \theta_1\theta_2 + \alpha\theta_2)$, see equation 1.6.11 in chapter 1, an easy computation gives for the first return map:

$$\begin{aligned} y_1(n+1) &= f(y_1(n)) \\ &= \frac{\theta_1\alpha\beta y_1(n)}{\theta_1\alpha\beta - \delta y_1(n)} \end{aligned}$$

therefore

$$f'(y_1) = \left(\frac{\theta_1\alpha\beta}{\theta_1\alpha\beta - \delta y_1}\right)^2 \tag{6.2.3}$$

so that $|f'(0)| = 1$ and $\forall y_1$ $(-\theta_1 < y_1 < 0)$ then $|f'(y_1)| < 1$, the point $y_1 = 0$ is stable and globally stable.

More generally, if we define $\rho = 1$, and $\sigma = \frac{-\delta}{\theta_1\alpha\beta}$ the first return map can be written

with the general form:

$$\begin{aligned} f(y_1, u) &= \frac{\rho y_1}{\sigma y_1 + 1} \\ f'(y_1, u) &= \frac{\rho}{(\sigma y_1 + 1)^2} \end{aligned}$$

and we have the following proposition given in [31]

Proposition 6.1. *For a first return map of the form:*

$$\frac{\rho y}{\sigma y + 1} \tag{6.2.4}$$

then if $\rho \leq 1$, the system is stable around $y = 0$; if $\rho > 1$ then there exists a stable limit cycle for $y = \frac{\rho - 1}{\sigma}$.

This result will be useful in the next section.

6.3 The genetic oscillator with control: first approach

To be able to control the system, we have first to define its outputs, i.e. the measurements that are available. Very often, classical control theory assumes that a measure of the real value x_1 or x_2 can be obtained, with a good precision, and maybe some noise. For genetic regulatory networks, the measurements are often of qualitative or even Boolean, indicating only if a gene is strongly or weakly expressed (see [41] for a review of experimental methods, such as microarrays or Western blots; see also [28]). In the framework of PWA systems, this means that we only know the domain (B_{ij}) where the variables are, and not their precise values.

Knowing that, a classical control (for example proportional to the variable) is not possible, and we can only fix a constant value for this control within each domain. This value may vary from domain to domain. We will suppose that the biologist is able to control, to some extent, the synthesis rate of one gene. It is a classical procedure, that can be done via the construction of a plasmid or genetic modifications of the DNA strain to be able to act on the expression of the gene via some inducer. The goal of the control will be to obtain sustained oscillations, i.e a periodic behaviour, as in [21].

Therefore if the control is on the synthesis rate of the first gene (for example), the new system is:

$$\begin{cases} \dot{x_1} = & u(B_{ij})k_1 s^-(x_2, \theta_2) - \gamma x_1 \\ \dot{x_2} = & k_2 s^+(x_1, \theta_1) - \gamma x_2 \end{cases}$$

where B_{ij} is one of the four domains defined above. The value of the control depends only on the domain; in two domains B_{01} and B_{11}, the control is not active (has no action on the system) because the function $s^-(x_2, \theta_2)$ cancels. Therefore it is *not possible* to control the system in these two regions. The control is only active in the two other regions, and may take two values; moreover, it should be positive. One important remark is that, even with a control, the system is still PWA. The control u is u_1 in B_{00} and u_2 in B_{10}.

For this first approach, we compute the first-return map from one boundary of a domain inside itself. The system being linear and decoupled (diagonal) with each domain, the computations are easy and have been done many times. We suppose that the control is such that $\theta_1 < \frac{u_1 k_1}{\gamma}$ and $\theta_2 < \frac{u_2 k_2}{\gamma}$, to have an oscillatory behaviour (see Assumption (2)).

After a simple change of variables $t' = \gamma t$, $y_1 = x_1 - \theta_1, y_2 = x_2 - \theta_2$, the first return map starting from the boundary $y_1 = y_1^0 < 0, y_2 = 0$ is given by:

$$f(y_1, u_1, u_2) = \frac{\theta_2\theta_1}{\beta + \theta_2 - \frac{\beta\theta_1}{au_2 + \frac{(au_2-\theta_1)\beta}{\frac{\theta_2(au_1-\theta_1)}{(au_1-\theta_1)-y_1(n)} - \theta_2 - \beta}}} - \theta_1 \tag{6.3.1}$$

with $a = \frac{k_1}{\gamma}$ and can be simplified into

$$f(y_1, u_1, u_2) = \frac{\theta_1\beta y_1(au_2 - \theta_1)}{-au_2(\beta + \theta_2)y_1 + \beta\theta_1 au_1 - \beta\theta_1^2} \tag{6.3.2}$$

There will exist a limit cycle if $y_1 = y_1^* < 0$ for some y_1^*, therefore

$$\begin{aligned} f(y_1^*, u_1, u_2) &= y_1^* && (6.3.3)\\ &\Rightarrow \frac{\theta_1\beta y_1^*(au_2 - \theta_1)}{-au_2(\beta + \theta_2)y_1^* + \beta\theta_1 au_1 - \beta\theta_1^2} = y_1^* && (6.3.4)\\ &\Rightarrow \frac{u_2}{u_1} = \frac{\beta\theta_1}{\theta_1\beta + (\beta + \theta_2)y_1^*} && (6.3.5) \end{aligned}$$

This implies for the control that

$$\frac{u_2}{u_1} = \frac{\theta_1(-k_2 + \theta_2\gamma)}{\theta_1\theta_2\gamma - k_2 x_1^*}$$

We easily have the proposition:

Proposition 6.2. *The control should be such that $u_2 > u_1$. For a control such that $u_2 = \frac{\beta\theta_1 u_1}{\theta_1\beta + (\beta+\theta_2)y_1^*}$ then the system has a stable limit cycle around $y_1^* = \frac{\rho-1}{\sigma}$.*

Proof. The first-return map is:

$$\begin{aligned} f(y_1, u_1, u_2) &= \frac{\theta_1\beta(au_2 - \theta_1)y_1}{-au_2(\beta + \theta_2)y_1 + \beta\theta_1 au_1 - \beta\theta_1^2} && (6.3.6)\\ &= \frac{\frac{\theta_1\beta(au_2-\theta_1)}{\beta\theta_1 au_1 - \beta\theta_1^2} y_1}{\frac{-au_2(\beta+\theta_2)}{\beta\theta_1 au_1 - \beta\theta_1^2} y_1 + 1} && (6.3.7) \end{aligned}$$

of the form

$$\frac{\rho y}{\sigma y + 1} \tag{6.3.8}$$

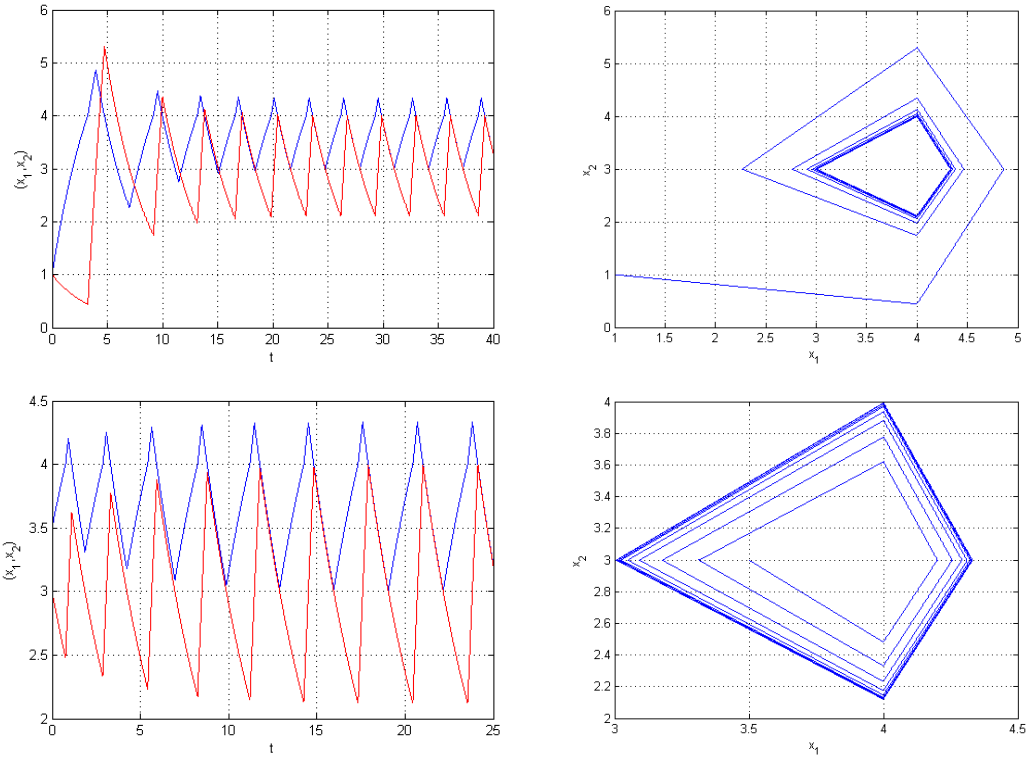

FIGURE 6.3.1: The simulations from two initial conditions with control u_1, u_2 verifying equality (6.3.5). A stable limit cycle with $x_1^* = 3$

with

$$\rho = \frac{\theta_1\beta(au_2 - \theta_1)}{\beta\theta_1 au_1 - \beta\theta_1^2} \tag{6.3.9}$$

$$\sigma = \frac{-au_2(\beta + \theta_2)}{\beta\theta_1 au_1 - \beta\theta_1^2} \tag{6.3.10}$$

From the above proposition (6.2.4), a stable limit cycle will exist if $\rho > 1$, that is

$$\frac{\theta_1\beta(au_2 - \theta_1)}{\beta\theta_1 au_1 - \beta\theta_1^2} > 1 \quad \Rightarrow \quad \theta_1\beta(au_2 - \theta_1) > \beta\theta_1 au_1 - \beta\theta_1^2 \tag{6.3.11}$$

$$\Rightarrow \quad \theta_1\beta a(u_2 - u_1) > 0 \tag{6.3.12}$$

$$\Rightarrow \quad u_2 > u_1 \tag{6.3.13}$$

□

With the parameters $k_1 = 2, k_2 = 4, \gamma_1 = \gamma_2 = 0.25, \theta_1 = 4, \theta_2 = 3, x_{01} = x_{02} = 1$, $u_1 = 0.8$, simulations are given Fig. (6.3.1).

6.4 The genetic oscillator with control: second approach

For this approach, we decide to introduce an additional threshold for one variable, that will permit to use a theorem in [23]. We recall this theorem (slightly adapted):

Assumption 3. Pairs of successive focal points are *aligned*, e.g. in two successive focal points, at most one coordinate changes.

Theorem 6.3. *Let $\mathcal{C} = \{a^0, a^1 \cdots a^{\ell-1}\}$ denote a sequence of regular domains which is periodically visited by the flow, and such that each domain a^i has a unique exiting direction s_i. Suppose that the focal points of $\mathcal{C}$ satisfy Assumption 3, i.e. they are aligned. Suppose also that all variables are switching at least once.*
Consider the first return map $\mathbf{T} : W^0 \to W^0$. Let $\lambda = \rho(D\mathbf{T}(0))$, the spectral radius of $D\mathbf{T}(0)$. Then, the following alternative holds:

i) if $\lambda \leqslant 1$, then $\forall x \in W^0$, $\mathbf{T}^n x \to 0$ when $n \to \infty$. The equilibrium is globally stable.

ii) if $\lambda > 1$ then there exists a unique nonzero fixed point $q = \mathbf{T}q$. Moreover, for every $x \in W^0 \setminus \{0\}$, $\mathbf{T}^n x \to q$ as $n \to \infty$: the unique limit cycle is globally stable for every solution starting outside the equilibrium.

Suppose that there are two distinct crossed thresholds in at least one direction, then the conclusion of ii) holds.

We now consider the same system as above

$$\begin{cases} \dot{x_1} = & u(B_{ij})k_1 s^-(x_2, \theta_2) - \gamma x_1 \\ \dot{x_2} = & k_2 s^+(x_1, \theta_1) - \gamma x_2 \end{cases}$$

with a control of the synthesis rate of the first variable, but we define now the control u in a more qualitative way, with a second threshold $\theta_2' < \theta_2$. We design control u as:

$$u = (\alpha + (1-\alpha)s^-(x_2, \theta_2'))(1 + \delta s^+(x_1, \theta_1)))(1 - \epsilon s^-(x_2, \theta_2')s^+(x_1, \theta_1))$$

with $0 < \alpha < 1$ and $\delta > 0, 0 < \epsilon < 1$, this control is positive. Moreover we choose $(1+\delta)(1-\epsilon) = 1$.

We remark that u may take at most three different values depending on the domain: $\alpha, \alpha(1+\delta), 1$. We choose $\alpha < 1$ and $\alpha(1+\delta) = 1$. With this choice, u has the value $u = 1$ (no control), or a low value α. Therefore two values of the control (low control or no control) are only necessary. Moreover we suppose that the usual assumptions

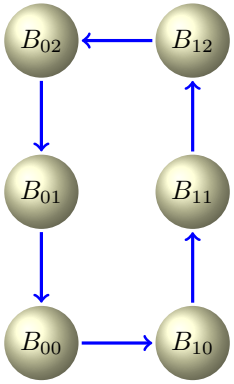

FIGURE 6.4.1: The transition graph for the controlled system

for an oscillatory behaviour are fulfilled (see Assumption (2) and that α is such that $\alpha k_1/\gamma < \theta_1$ to place the focal point of B_{01} in the domain B_{00}.

We now use the above theorem to show that, in our example, there is a unique stable limit cycle. We compute the focal point associated with each domain (there is now 6 domains because one more threshold for x_2).

In the table, we compute the focal point associated with each domain. We have multiplied everything by γ to simplify.

$\mathcal{C}:$ 00	10	11	12	02	01
k_1	k_1	$k_1\alpha(1+\delta)$	0	0	$k_1\alpha$
0	k_2	k_2	k_2	0	0

(6.4.1)

A cycle $\mathcal{C}$ involving all regular domains exists for any parameter set satisfying the specified constraints. Moreover, any pair of successive focal points only differ in the switching direction, i.e. assumption (3) is verified. The graph of transition of the system with control is given Fig. 6.4.1. Hence, we may apply theorem 6.3, and since θ_2 and θ_2' are both crossed in $\mathcal{C}$, we conclude that there exists a unique stable periodic orbit attracting all initial conditions, as shown on the simulation of Fig.6.4.2.

This control is very robust: it takes only one low value under the value $u = 1$ (no control). Moreover, the precise value of the second threshold θ_2' and of parameters α, δ for the design of the control have very light constraints (only $\theta_2' < \theta_2, 0 < \alpha < 1, \delta > 0, 0 < \epsilon < 1$).

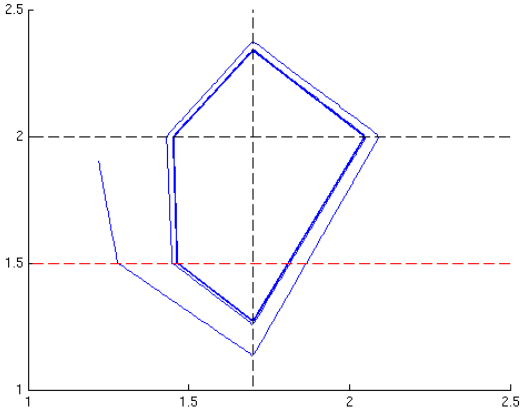

FIGURE 6.4.2: The resulting limit cycle (parameters $k_1 = 3, k_2 = 4, \gamma = 1, \theta_1 = 1.7, \theta_2 = 2, \theta_2' = 1.5, \alpha = 0.5, \delta = 1, \epsilon = 0.5$)

6.5 Qualitative control for a negative ODE loop

We now consider a slightly different problem: the model is given by an ODE, and we try to control it in a qualitative way. The degradation rates are no more equal.

We take a negative loop with two genes as above, given by

$$\begin{cases} \dot{x_1} = & k_1\, h^-(x_2, \theta_2, n) - \gamma_1 x_1 \\ \dot{x_2} = & k_2\, h^+(x_1, \theta_1, n) - \gamma_2 x_2 \end{cases} \tag{6.5.1}$$

where h^+ and h^- are the increasing or decreasing Hill functions: $h^+(x, \theta, n) = x^n/(x^n + \theta^n)$, $h^-(x, \theta, n) = \theta^n/(x^n + \theta^n)$.

This system has a unique positive equilibrium x_1^*, x_2^*, under the classical assumptions

$$\theta_1 < \frac{k_1}{\gamma_1}; \quad \theta_2 < \frac{k_2}{\gamma_2}$$

Without control ($u = 1$), this equilibrium is globally asymptotically stable with an oscillatory behaviour, as we have seen above (see also Fig. (6.5.1)). To design a control that cancels these oscillations is very easy, it is enough to takes

$$u = \alpha / h^-(x_2, \theta_2, n)$$

this control transforms the system into a linear stable system (α is a positive parameter), and moreover is positive. But to implement it, we need to measure precisely the state x_2.

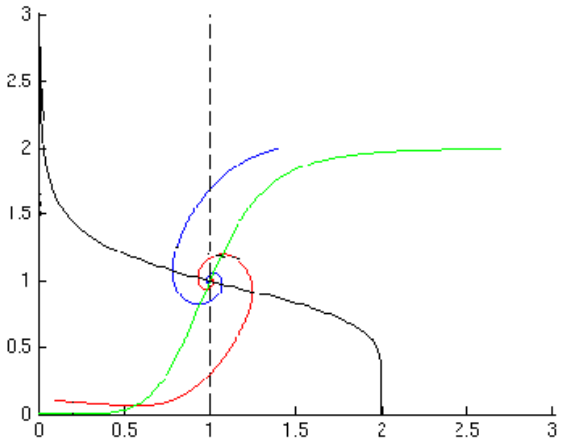

FIGURE 6.5.1: Oscillations without control (parameters $k_1 = 1, k_2 = 1, \gamma_1 = 0.5, \gamma_2 = 1, \theta_1 = 1, \theta_2 = 1, n = 6$)

Now, we are looking for a qualitative control u, depending on simple regions, such that the controlled system:

$$\begin{cases} \dot{x_1} = & u\ k_1\ h^-(x_2, \theta_2, n) - \gamma_1 x_1 \\ \dot{x_2} = & k_2\ h^+(x_1, \theta_1, n) - \gamma_2 x_2 \end{cases} \tag{6.5.2}$$

has no oscillatory behaviour around the equilibrium. We design the control u with two values: a "low" value u^- and a "high" value u^+, such that $u^- < 1 < u^+$ (of course, $u = 1$ corresponds to no control). The control will change if x_1 is smaller or greater than its equilibrium x_1^*.

$$if \quad x_1 > x_1^*, u = u^-; \quad if \quad x_1 < x_1^*, u = u^+$$

On the boundary, the control is not defined; the solution of the system is defined with the Filippov solution, see in chapter 1. The goal is to obtain a stable sliding mode on the line x_1^*.

First, to simplify, we make the assumption that u^- is small enough so that the first nullcline of the system with u^- is completely contained in the half-space $x_1 < x_1^*$. The condition for that is $u^- k_1/\gamma_1 < x_1^*$.

Then we study the existence (or not) of a sliding mode along the line x_1^*. The study is elementary, and done with a simple phase plane analysis of the sign of vector fields between the nullclines. On the figures Fig. (6.5.1) and Fig. (6.5.2), we have drawn the second nullcline in green (it does not depend on the control) and the three nullclines in

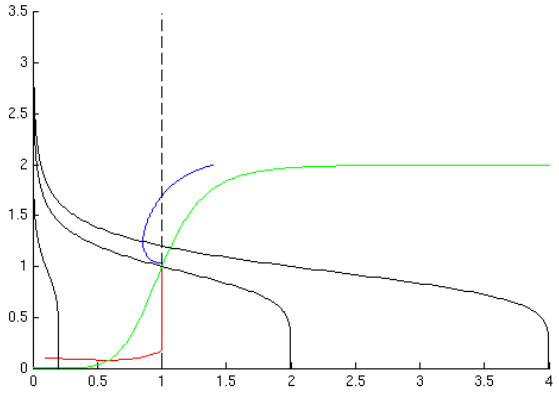

FIGURE 6.5.2: Sliding mode with control ; the three black curves are the three nullclines for $u = u^-, 1, u^+$. Parameters for control are $u^- = 0.1, u^+ = 2$

black, for $u^-, u = 1, u^+$. In the left half space $x_1 < x_1^*$, the control is u^+, and in the right half-space $x_1 > x_1^*$, the control is u^-. We note P the point at the intersection of x_1^* and of the first nullcline for u^+, then there is a sliding mode on x_1^* below this point P. Above P, on the boundary, the two vector fields have the same sign and cross from right to left. Moreover, any trajectory in the plane will end up on the sliding mode line.

On the sliding mode itself, we have $x_1 = x_1^*$, and the equation for x_2 is:

$$\dot{x_2} = k_2 \, h^+(x_1^*, \theta_1, n) - \gamma_2 x_2$$

which is a simple affine system converging towards the equilibrium. Therefore the sliding mode converges towards the desired reference equilibrium. Fig. (6.5.3) shows a zoom of the phase portrait towards the equilibrium.

We see that the oscillations of the controlled system are canceled. The same method is applicable in n dimensions for a negative loop, but the precise proof has to be done.

6.6 Conclusion

Two different solutions for creating a limit cycle in a two genes system are given. Many extensions of this work are possible. The first one, as indicated in the first section, consists in a generalization of the result when the degradation rates γ_i are distinct. This is easy, specially in the second approach, because the theorem we use is also valid for distinct degradation rates. Then we qualitatively control an ODE negative loop.

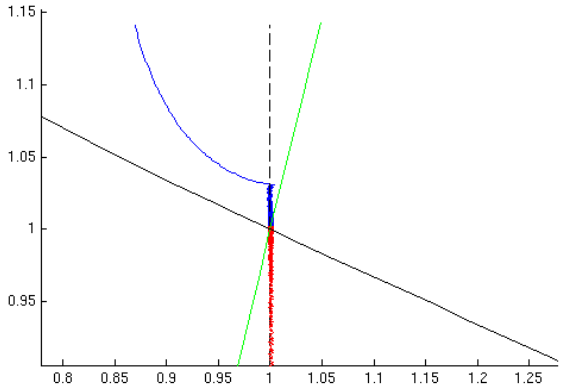

FIGURE 6.5.3: Sliding mode with control, zoom around the equilibrium

A more interesting generalization would be to consider an oscillator in a higher dimension: three for example, as in the famous example of the repressilator [22]. No theoretical obstacle is visible to apply our technique. The problem in that case is that, in dimension three, there is already a stable limit cycle without control in the PWA case. The control should be used to change the limit cycle (modify the amplitude or the period) or suppress it. Further work is needed to solve this interesting problem.

To conclude, we think that problems of quantitative control (control within a domain) are a promising area of investigation, amenable to experimental outlets.

Chapter 7

Conclusions et perspectives

Les réseaux biologiques sont composés généralement d'une partie génétique et d'une partie métabolique en interaction. La partie génétique représente des séquences d'ADN qui produisent des protéines. La partie métabolique représente des réactions chimiques catalysées par des enzymes (les réactions enzymatiques). Les enzymes sont des protéines codés par des gènes (partie génétique). Nous avons traité dans cette thèse le problème de la modélisation, de la réduction et l'analyse de modèles de tels réseaux biologiques qui sont généralement avec différentes échelles de temps ; nous traitons aussi le problème de contrôle qualitative des petits systèmes des réseaux génétiques.

Pour mieux comprendre les modèles mathématiques que nous avons abordés dans cette thèse, nous avons commencé au début dans le chapitre 1 par présenter quelques notions de la biologie en expliquant le dogme central de la biologie moléculaire, puis les principes de base que l'on a utilisé pour la modélisation mathématique des réseaux de régulation génétique en utilisant des équations différentielles continues ou continues par morceau au cours du temps. Nous avons vu que la modélisation par des fonctions steps à la place des fonction de Hill peut simplifier l'étude et l'analyse des systèmes de très grande dimension.

Nous avons traité dans le deuxième chapitre de la modélisation et l'analyse des réactions enzymatiques et du couplage métabolique-génétique. Nous avons pris un modèle très connu, de Michaelis-Menten, complet, sans le réduire en utilisant l'hypothèse du quasi équilibre qui ne marche que sous certaines conditions qui ne sont pas toujours vérifiés. Nous avons étudié en utilisant des techniques des systèmes monotones et compartimentaux, la stabilité globale de l'équilibre du système fermé complet. Le système ouvert n'a jamais été étudié avant, et pour ce système nous avons remarqué qu' il peut ne pas avoir

un équilibre et nous avons appliqué également des techniques de systèmes monotones pour montrer que si l'équilibre existe alors il est globalement stable.

Nous avons étudié aussi le cas général d'une chaine de réactions enzymatiques. Les réactions enzymatiques sont généralement en interaction ou en liaison avec des parties génétiques et forment ainsi ce qu'on appelle des réseaux biologiques. Ces modèles des réseaux biologiques sont généralement non linéaires, de grandes dimensions et très complexes à analyser. La dynamique des système métaboliques est souvent très rapide en comparant avec la dynamique du système génétique. Dans la deuxième étape dans ce chapitre nous avons formé différentes types de modèles du couplage des chaines des réactions métaboliques avec une partie génétique et nous avons montré comment on peut les réduire en se basant sur cette différence d'échelles de temps, utilisant le théorème de Tikhonov, et en appliquant toujours les même techniques de systèmes monotones et compartimentaux. Nous concluons que si l'équilibre de la partie rapide s'échappe à l'infini alors le modèle réduit devient invalide.

Pour ce chapitre il reste à faire l'étude et l'analyse de chaque système réduit. Nous considérons toujours pour le couplage métaboliques-génétiques dans ce chapitre que le produit final de la chaine des réactions enzymatiques inhibe ou active la production de l'enzyme en passant par le gène, plusieurs autres systèmes peuvent être possible et peuvent être aussi étudié : par exemple le produit final permet aussi l'inhibition de l'enzyme sans passer par le gène. Il serait intéressant de comparer l'effet pour les deux cas : inhibition directe et une inhibition par le gène.

Les produits intermédiaires dans une chaine de réactions enzymatiques peuvent inhiber aussi la production d'une enzyme donc on peut prendre d'autres systèmes où on va choisir un produit intermédiaire au lieu de considérer le produit final pour influencer la production d'un enzyme.

Dans le troisième chapitre, nous avons appliqué les mêmes techniques de systèmes monotones pour étudier un modèle général d'expression d'un gène. Le modèle seul de la première étape (la transcription) ou de la deuxième étape (la traduction) du processus d'expression d'un gène est monotone, dont nous avons pu facilement étudier et analyser chaque modèle séparément utilisant les mêmes techniques. Le modèle couplé de la transcription et la traduction n'est pas monotone (et difficile à analyser). L'étude et la réduction de ce modèle général pour l'expression d'un gène pour la protéine est donné dans [4]. Le modèle réduit est donné par:

$$
\begin{aligned}
z &= l\,\frac{k_c}{k_t}\,d_0\,\frac{p}{p+K_1} + p + d_0\,\frac{p}{p+K_1}\\
R_0 &= h\frac{k_w}{k_t'}\frac{q\,r}{r+K_2} + r + \frac{q\,r}{r+K_2}\\
\dot{z} &= k - k_p\,p(z)\\
\dot{q} &= k_c d_0\,\frac{p(z)}{p(z)+K_1} - k_m\,(q - \frac{q\,r(q)}{r(q)+K_2})\\
\dot{s} &= k_w\frac{q\,r(q)}{r(q)+K_2} - k_s\,s
\end{aligned}
\tag{7.0.1}
$$

Nous supposons dans cet article [4] que le ARNm libre se dégrade seul; si comme nous l'avons fait dans le chapitre 4 on suppose que si la forme libre de ARNm se dégrade alors la forme liée se dégrade aussi avec le même taux et libère la même quantité du ribosome dans le complexe, ce modèle réduit devient:

$$
\begin{aligned}
z &= l\,\frac{k_c}{k_t}\,d_0\,\frac{p}{p+K_1} + p + d_0\,\frac{p}{p+K_1}\\
R_0 &= h\frac{k_w}{k_t'}\frac{q\,r}{r+K_2} + r + \frac{q\,r}{r+K_2}\\
\dot{z} &= k - k_p\,p(z)\\
\dot{q} &= k_c d_0\,\frac{p(z)}{p(z)+K_1} - k_m\,q\\
\dot{s} &= k_w\frac{q\,r(q)}{r(q)+K_2} - k_s\,s
\end{aligned}
\tag{7.0.2}
$$

Une comparaison avec les résultats obtenu par Kremling (voir [42]) peut être intéressante, et une interprétation du point de vue biologique est aussi intéressante. L'équation du ARNm: $\dot{q} = k_c d_0\,\frac{p(z)}{p(z)+K_1} - k_m\,q$, est plus ou moins la même, mais en fait, on voit bien que dans notre système réduit, $p(z)$ dépend de la concentration totale du promoteur. C'est encore plus clair en regardant par exemple la solution analytique du l'équation algébrique $z = l\,\frac{k_c}{k_t}\,d_0\,\frac{p}{p+K_1} + p + d_0\,\frac{p}{p+K_1}$. Idem pour l'équation de la protéine qui est plus ou moins la même.

Nous avons vu dans [4] que le polymérase va aller vers un équilibre z^* donc on peut écrire:

$$
\begin{aligned}
R_0 &= h\frac{k_w}{k_t'}\frac{q\,r}{r+K_2} + r + \frac{q\,r}{r+K_2}\\
\dot{q} &= k_c d_0\,\frac{p(z^*)}{p(z^*)+K_1} - k_m\,q\\
\dot{s} &= k_w\frac{q\,r(q)}{r(q)+K_2} - k_s\,s
\end{aligned}
\tag{7.0.3}
$$

Nous avons vu dans le chapitre 1 que le modèle classique d'expression d'un gène pour la protéine est donné par:

$$\begin{aligned} \dot{q} &= kh(G) - k_m q \\ \dot{s} &= \alpha q - k_s s \end{aligned} \tag{7.0.4}$$

$h(G)$ est une fonction de régulation, et G un facteur de transcription (une autre protéine). Nous avons vu aussi que ce modèle classique peut être réduit en une seule équation qui ne dépend ni de la polymérase ni de la protéine ni du site promoteur qui n'ont aucun rôle dans ce modèle. Une comparaison avec notre modèle est intéressante : par exemple l'équation classique de la protéine est linéaire en q alors que dans notre modèle réduit on voit très bien qu'elle est non linéaire en q.

Dans le quatrième chapitre, nous considérons un modèle en boucle où la polymérase permet la transcription du gène de la polymérase. En se basant sur des valeurs de paramètre fournis par des biologistes, nous l'avons réduit à un modèle plus simple et monotone. Nous avons pu étudier et analyser ce système réduit en utilisant la théorie des systèmes monotones concaves (la matrice jacobienne est décroissante). L'étude du système réduit montre que le système complet peut avoir soit un seul point d'équilibre à l'origine qui est globalement stable ou bien il existe un autre point d'équilibre strictement positif stable et l'origine est localement instable. L'alternative entre ces deux cas dépend de la quantité totale de la concentration des ribosomes.

Le cinquième chapitre est la suite logique du chapitre précédent, nous avons étudié un modèle plus général de la machinerie génétique, en prenant le modèle étudié précédemment pour la polymérase et en le couplant avec un modèle pour la synthèse des ribosomes. Ce modèle est aussi monotone mais avec une matrice jacobienne n'est pas décroissante. Calculer explicitement (analytiquement) les valeurs des équilibres est très difficile, nous avons prouvé géométriquement en se basant sur des valeurs numérique des paramètres de ce modèle qu'il existe trois équilibres ; en fonction de ces valeurs numérique on obtient qu'il y a un équilibre à l'origine, un autre très proche de l'origine et l'équilibre qui reste est strictement positif et très loin de l'origine.

Nous avons appliqué des approximations pour réduire encore plus ce modèle en se basant sur ce que nous avons constaté (un équilibre très petit et une autre très grand), nous avons pu calculer les expressions pour les valeurs des équilibres et nous avons étudié la stabilité locale de chaque équilibre : nous avons conclu que l' origine est localement stable, l'équilibre qui est très proche de l'origine est instable et le troisième est localement

stable. Nous traitons très rapidement la stabilité globale de chaque équilibre et nous avons donné des conclusions mais il nous reste à rédiger les preuves. L'étude de la stabilité globale de chaque équilibre est possible comme dans le chapitre 4 en utilisant les même techniques des système monotone mais nous n'avons pas eu le temps pour le faire d'une manière approfondie et avec plus de précision.

Pour la machinerie génétique, il reste beaucoup de choses à faire: par exemple ces modèles que l'on a étudié dans le chapitre 4 et 5 ne sont pas isolés, plusieurs facteurs de régulations peuvent être ajoutés, par exemple la fonction du ppGpp qui permet l'inhibition de la production de ribosomes. Le modèle dans le chapitre 4, si on suppose aussi que le ribosome peut se dégrader et sans synthèse (inhibition par du ppGpp), va aller vers zéro alors que si on suppose qu'il y a synthèse des ribosomes (pas d'influence de ppGpp) alors on retrouve le modèle qu'on a étudié dans le chapitre 5. Il serait peut être intéressant de voir ce qui passe si on rajoute une autre variable ou une fonction pour les ppGpp dans le modèle dans le chapitre 5 pour avoir des commutations entre les deux cas; soit il y a trois équilibres ou tout va aller à zéro : peut être on peut avoir un cycle limite ?. On peut encore généraliser le modèle en le couplant aussi avec un modèle pour la synthèse des bulk protéines, et d'étudier le système réduit de ce modèle qui a été déjà proposé par Delphine Ropers.

Pour tous ces modèles qu'on a étudiés avant, nous avons utilisé les même techniques des systèmes monotones et compartimentaux pour la réduction du système et pour l'étude de la stabilité globale; malgré les modèles des réseaux biologiques qui changent, nous remarquons qu'on utilise les mêmes techniques qui restent toujours valables.

Dans le sixième chapitre, nous avons appliqué à la fin différents types de contrôles qualitatifs sur des modèles de petits réseaux génétique non linéaires. Le contrôle qualitatif ou par région est très intéressant en biologie car les techniques de contrôle classiques ne peuvent pas être appliquées. Ce chapitre est plus exploratoire, dans notre étude nous avons pris un modèle d'un oscillateur en dimension 2 où on essaye au début de faire le contrôle pour avoir un cycle limite au lieu d'un équilibre stable, mais par exemple il est possible de faire une généralisation en dimension n, surtout pour la deuxième approche de contrôle que l'on a appliquée.
Nous avons appliqué dans la deuxième étape le contrôle par mode glissant pour faire le contraire: empêcher le système de faire des oscillations et le forcer à converger vers son équilibre par mode glissant. Ces techniques de modes glissant peuvent être aussi appliquées à d'autres systèmes, voir par exemple [44].

Pour des système en dimensions trois ou plus, on peut avoir un cycle limite, prenons par exemple ce système en trois dimensions :

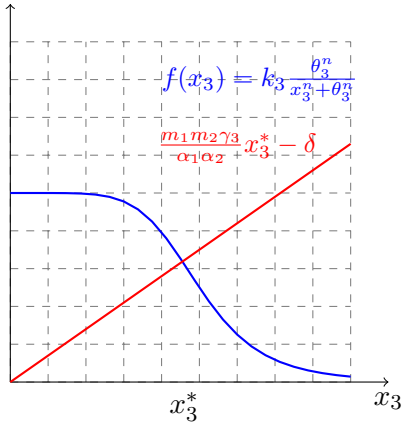

FIGURE 7.0.1: Un seul point d'équilibre

$$\begin{aligned} \dot{x_1} &= f(x_3) + \delta - m_1 x_1 \\ \dot{x_2} &= \alpha_1 x_1 - m_2 x_2 \\ \dot{x_3} &= \alpha_2 x_2 - \gamma_3 x_3 \end{aligned} \tag{7.0.5}$$

avec $m_1 = \gamma_1 + \alpha_1$, $m_2 = \gamma_2 + \alpha_1$, et $f(x_3) = k_3 \frac{\theta_3^n}{x_3^n + \theta_3^n}$ représente une fonction de Hill où x_3 inhibe la synthèse de la protéine x_1. A l'équilibre de ce système nous avons:

$$\begin{aligned} x_1^* &= \frac{f(x_3) + \delta}{m_1} \\ x_2^* &= \frac{\alpha_1 x_1}{m_2} \\ x_3^* &= \frac{\alpha_2 x_2}{\gamma_3} \end{aligned} \tag{7.0.6}$$

Donc on obtient cette égalité à l'équilibre:

$$f(x_3) = k_3 \frac{\theta_3^n}{x_3^n + \theta_3^n} = \frac{m_1 m_2 \gamma_3}{\alpha_1 \alpha_2} x_3^* - \delta \tag{7.0.7}$$

Le tracé de ces deux fonctions ($f(x_3)$ et $\frac{m_1 m_2 \gamma_3}{\alpha_1 \alpha_2} x_3^* - \delta$) est représenté dans la figure 7.0.1, donc le système admet un seul point d'équilibre.

La matrice jacobienne du système au point d'équilibre est donnée par:

$$\begin{pmatrix} -m_1 & 0 & f'(x_3^*) \\ \alpha_1 & -m_2 & 0 \\ 0 & \alpha_2 & \gamma_3 \end{pmatrix} \tag{7.0.8}$$

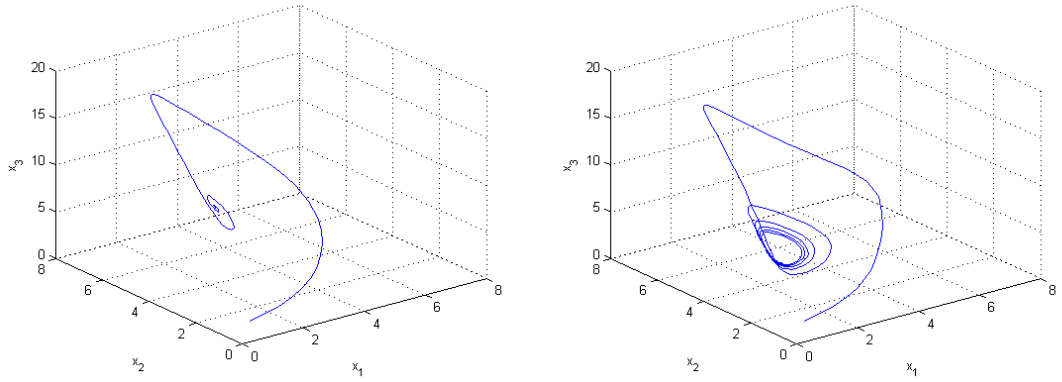

FIGURE 7.0.2: Simulations avec les valeurs de paramètre $k_3 = 3, m_1 = 0.25, m_2 = 0.25, \alpha_1 = 0.5, \alpha_2 = 0.7, \gamma_3 = 0.25, \theta_3 = 5$, $n = 3$ à gauche, $n = 10$ à droite, et avec la condition initiale $x_{01} = 1, x_{02} = 1, x_{03} = 0.5$

Le polynôme caractéristique du système linéarisé autour du point d'équilibre est donné par:

$$\lambda^3 + (m_1 + m_2 + \gamma_3)\lambda^2 + ((m_1 + m_2)\gamma_3 + m_1 m_2)\lambda + m_1 m_2 \gamma_3 + n\frac{x_3^{*n-1}\theta_3^n}{x_3^{*n-1} + \theta_3^n} = 0$$

Alors pour que ce système soit localement stable il suffit que:

$$(m_1 + m_2 + \gamma_3)((m_1 + m_2)\gamma_3 + m_1 m_2) - m_1 m_2 \gamma_3 - n\frac{x_3^{*n-1}\theta_3^n}{x_3^{*n} + \theta_3^n} > 0$$

ce qui implique

$$(m_1 + m_2)(\gamma_3^2 + (m_1 + m_2)\gamma_3 + m_1 m_2) - n\frac{x_3^{*n-1}\theta_3^n}{x_3^{*n} + \theta_3^n} > 0$$

L'équilibre est localement instable si nous prenons par exemple n très grand, voir les résultats de simulations dans la figure 7.0.2 en prenant $n = 3$ et après, $n = 10$. Nous remarquons que si n est grand l'équilibre devient instable et que l'on a obtenu un cycle limite.

Donc on peut par exemple faire la même chose qu'avant d'appliquer un contrôle pour l'expression du gène de la protéine x_1 pour stabiliser le point d'équilibre dans le cas instable (n très grand). Le système avec le contrôle est donné par:

$$\begin{aligned} \dot{x_1} &= uf(x_3) + \delta - m_1 x_1 \\ \dot{x_2} &= \alpha_1 x_1 - m_2 x_2 \\ \dot{x_3} &= \alpha_2 x_2 - \gamma_3 x_3 \end{aligned} \tag{7.0.9}$$

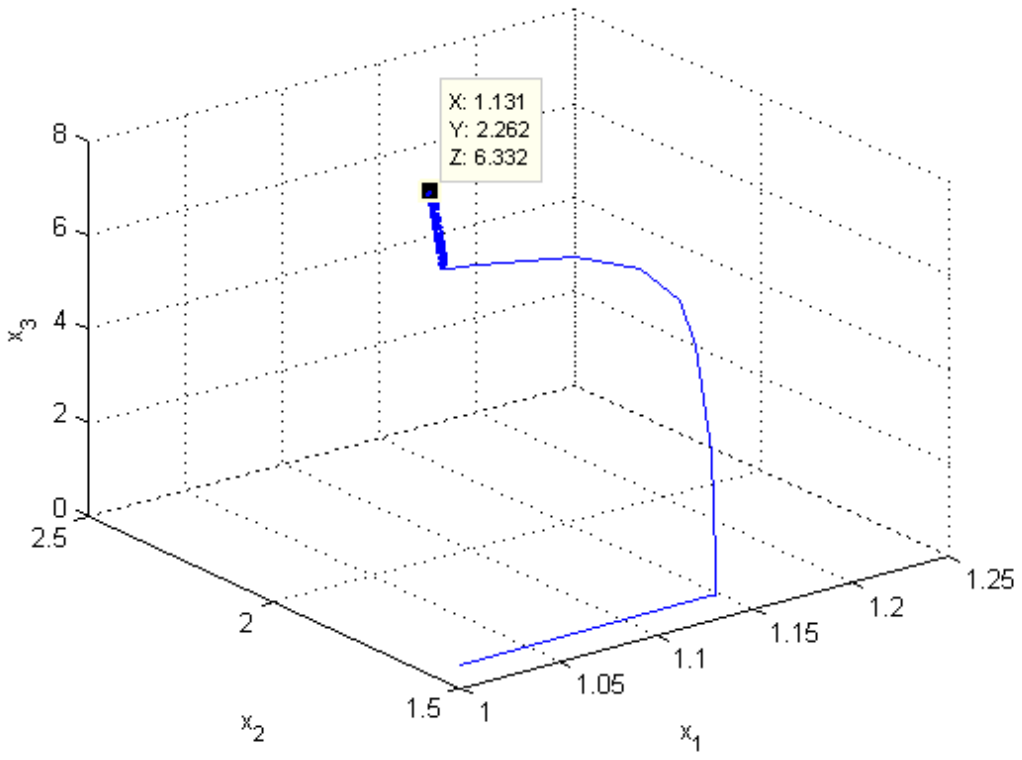

FIGURE 7.0.3: Le résultat de simulation en prenant un contrôle par modes glissants pour stabiliser le point d'équilibre instable, conditions initiales $x_{01} = 1, x_{02} = 1.5, x_{03} = 0.5$.

Alors pour $n = 10$, $u = 1$, et avec ces valeurs numériques $k_3 = 3, m_1 = 0.25, m_2 = 0.25, \alpha_1 = 0.5, \alpha_2 = 0.7, \gamma_3 = 0.25, \theta_3 = 5$, $n = 10$, $\delta = 0.02$, on trouve ces valeurs numériques pour l'équilibre $((x_1^*, x_2^*, x_3^*) = (1.13, 2.26, 6.32))$. Alors il sera intéressant aussi d'appliquer le contrôle par région et par modes glissants pour stabiliser ce point d'équilibre instable. Par exemple on peut prendre une valeur de contrôle très grande si $x_1 < 1.13$ et un contrôle très petit si $x_1 > 1.13$ pour avoir un mode glissant vers l'équilibre. Prenons par exemple $u = 10$, si $x_1 < 1.13$, et $u = 0.1$, si $x_1 > 1.13$, le résultat de simulation est donné dans la figure 7.0.3. On voit très bien qu'on obtient un mode glissant sur la surface $x_1(t) - x_1^* = 0$, et qui converge vers l'équilibre.

Une preuve formelle reste à écrire.

Une généralisation à des boucles négatives de plusieurs gènes est possible.

En perspective générale, nous pensons que ces techniques de contrôle qualitatif par région sont très prometteuses en biologie.

Appendix A

Monotone And Compartmental Systems

A.1 Monotone Systems

Monotone systems form an important class of dynamical systems, and are particularly well adapted to mathematical models in biology ([60]), because they are defined by conditions related to the signs of Jacobian matrix. Such a sign for one element traduces the fact that some variable will contribute positively to the variation of some other variables, and this kind of qualitative dependence is very frequent in biological models. The reader may consult the references [58] for a review or an exhaustive presentation of the theory of monotone systems.

In summary, if the system is cooperative, then the flow preserves the partial order of trajectories in $\Re^n$ (the flow is monotone). To clarify consider for example an autonomous differential system with only 2-dimensional:

$$\dot{x} = f(x) \tag{A.1.1}$$

where, $x \in \Re^2$ and $f_i : \Re^2 \to R$.

Therefore if the system is monotone this means that if $x_{01} < x_{02}$, then $x(t, x_{01}) < x(t, x_{02}) \ \forall \ t$, with:

- $x(t, x_{01})$: corresponds to the evolution with respect to time starting from the initial condition x_{01}.
- $x(t, x_{02})$: The evolution with respect to time starting from the initial condition x_{02}.

The phase space in this case is given in figure A.1.1:

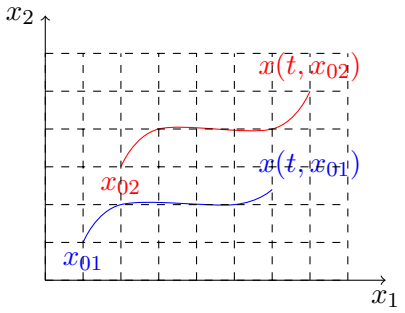

FIGURE A.1.1: Phase space in $\Re^2$ from two different initial conditions x_{01}, x_{02} and in the case that the system is monotone

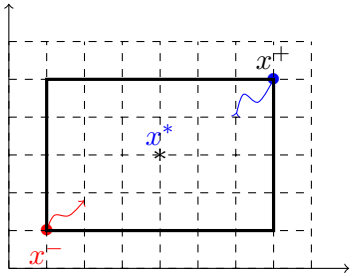

FIGURE A.1.2: Property of cooperative (or monotone) systems. The whole hyperrectangle built with these two points is invariant, and all the trajectories initiating in this rectangle converge toward the equilibrium

Cooperativity is easy to check by looking at the signs of the elements of the Jacobian matrix, that should verify

$$\frac{\partial f_i}{\partial x_j}(t,x) \geq 0 \quad \forall i \neq j$$

These systems have a strong tendency to converge to the set of their equilibria ([58]). It can be shown that almost any solution converges to the set of equilibria except a set of zero measure. In particular, there are no stable periodic solutions. For more precise theorems, see [58]. Here we only need a simple proposition, easily deduced from Proposition 2.1 p. 34 of [58]. The system is defined on a convex set X.

Proposition A.1. *Let us suppose that only one equilibrium x^* exists in X; if moreover it exists two points x^+,x^- in X such that $f(x^+) \leq 0$ and $f(x^-) \geq 0$, with $x^- < x^* < x^+$, then the hyperrectangle built by the two points x^-, x^+ is invariant, and every solution in this rectangle converges toward the equilibrium point, see figure A.1.2:*

A.2 Invariance of the Positive Orthant

The positive orthant is invariant if: whenever $x_{i0} \geq 0$, then $x_i(t; x_0) \geq 0$ for all $t \geq 0$ ($i = 1, \ldots, n$). The following condition guarantees *invariance of the positive orthant*

$$x_i = 0 \quad \Rightarrow \quad f_i(x) \geq 0, \quad i = 1, \ldots, n, \tag{A.2.1}$$

which means that, at the boundary of the positive orthant, the vector field is either zero or points towards the interior of the orthant, thus preventing the variables to decrease to negative numbers [43].

A.3 Matrices and Compartmental systems

We introduce the notion of irreducibility of a matrix, we give one of the possible definition, which will be used in theorem 2.2.

Definition A.2. Irreducible Matrix
A matrix is irreducible if its graph is strongly connected (there is a directed path from any compartment to any other compartment).

Let us now give a few reminders about compartmental systems (see[39]). This kind of models describes the dynamics of n -compartments interconnected by links with fluxes of matter. The overall equation is written by making a global mass balance between inputs and outputs of each compartment. The definition of a compartmental matrix is the following:

Definition A.3. Compartmental Matrix

Matrix J is a compartmental $(n \times n)$ matrix if it satisfies the following three properties ([39]):

$$J_{ii} \leq 0 \quad for \quad all \quad i, \tag{A.3.1}$$

$$J_{ij} \geq 0 \quad for \quad all \quad i \quad \neq j, \tag{A.3.2}$$

$$-J_{jj} \geq \sum_{i \neq j} J_{ij} \quad for \quad all \quad j \tag{A.3.3}$$

Note that, if J is a variable Jacobian matrix, J_{ij} can in general depend on $x_k, k = 1 \ldots n$ which are the concentrations in each compartment. A common case is when J_{ij}, flow of compartment j in the compartment i, depends only on x_j (thus on the concentration of the initial compartment). This is not the case in our systems. There are also some theorems on the stability of linear and nonlinear compartmental systems (see [39]).

We recall some definitions and properties concerning output, see [39, p. 47] and [3].

Definition A.4. Fully outflow connected network A compartment x_i is outflow (output) connected if there is a path $x_i \to x_j \to ... \to x_l$ from x_i until a compartment x_l with an outflow to the exterior of the system. The network is fully outflow connected if all compartments are outflow connected.

The following proposition is in [39, p. 52].

Proposition A.5. *Invertibility of a compartmental matrix*
A compartmental matrix is regular if and only if the associated network is fully outflow connected.

Intuitively, it means that the system has no traps where the flows accumulate (see [39]). We recall that in this case the matrix has eigenvalues with negative real parts [39, p. 51], and the associated linear system is asymptotically stable.

Now we introduce the notion of irreducibility of a matrix, we give one of the possible definition, which will be used in theorem 2.2.

Definition A.6. Irreducible Matrix
A matrix is irreducible if its graph is strongly connected (there is a directed path from any compartment to any other compartment).

A.4 Theorem on monotone and concave systems

This theorem is a corollary of Corollary 3.2 of [55], where we include the fact that the solutions are bounded.

We note $\mathring{R}^n_+$ the interior of the nonnegative orthant. The inequalities between vectors or matrices are term by term. All the functions involved are supposed regular enough (at least C^1) in R^n_+. We consider the differential system $\dot{x} = F(x)$, with $F(0) = 0$. The two assumptions are:

(Monotonicity) Jacobian matrix $J(x) = DF(x)$ is off-diagonal nonnegative, and irreducible for $x \in \mathring{R}^n_+$

(Concavity) Jacobian matrix $J(x)$ is decreasing: if $0 < x < y$, then $DF(x) \gneqq Df(y)$.

Then there is an alternative:

* either the trivial equilibrium 0 is locally linearly asymptotically stable (that can be computed with the Jacobian matrix $DF(0)$), then 0 is globally stable in the nonnegative orthant.

* or the trivial equilibrium is linearly unstable, and there exists a unique positive equilibrium x^* which is globally stable in the nonnegative orthant without the equilibrium 0.

We remark that we do not have to compute explicitly the value of the positive equilibrium.

Appendix B

The Frobenius-Perron theorem

B.1 The Frobenius-Perron theorem

In this Appendix we focus on the results given in [43] for positive linear systems. Let's consider this system :

$$\dot{x} = Ax \tag{B.1.1}$$

The main result of the Frobenius-Perron theorem is that for a matrix A, all of whose elements are strictly positive, there is an eigenvalue of largest absolute value and this eigenvalue is in fact positive and simple. Furthermore, there is a positive eigenvector corresponding to this positive eigenvalue. The Frobenius-Perron theorem is:

Theorem B.1. *If $A > 0$, then there exists a real eigenvalue $\lambda_0 > 0$ and a positive eigenvector $x_0 > 0$ such that*

- $Ax_0 = \lambda_0 x_0$;
- *if $\lambda_i \neq \lambda_0$ is any other eigenvalue of A, then $|\lambda_i| < \lambda_0$;*
- *λ_0 is an eigenvalue of geometric and algebraic multiplicity* 1.

A positive linear system is a linear system in which the state variables are always positive (or at least nonnegative) in value. A matrix $A \in \Re^{n\times n}$ is called a Metzler matrix if $a_{ij} \geq 0$ for all $j \neq i$; this means that all nondiagonal elements are nonnegative.

We say that the system (B.1.1) is positive if A is a Metzler matrix. Metzler matrices are obviously closely related to nonnegative matrices. Suppose A is a Metzler matrix. Then for some suitable constant $c > 0$, the matrix $P = cI + A$ is a nonnegative matrix. In particular, we have this Theorem:

Theorem B.2. *Let A be a Metzler matrix. Then there exists a real eigenvalue μ_0 and a non-negative eigenvector $y_0 \geq 0$ such that*

- $Ay_0 = \mu_0 y_0$*;*
- *if $\mu_i \neq \mu_0$ is any other eigenvalue of A, then $Re(\mu_i) < \mu_0$.*

Appendix C

Tikhonov's Theorem

C.1 Tikhonov's Theorem [66]

Consider this system:

$$\Sigma^{\epsilon} \begin{cases} \dfrac{dx}{dt} = f(x, z, \epsilon) \\ \epsilon \dfrac{dz}{dt} = g(x, z, \epsilon) \end{cases} \tag{C.1.1}$$

If theses two assumptions are satisfied

Assumptions 1. denote by $z = \rho(x)$ the root of the equation $g(x, z, 0) = 0$, we assume that $\rho(x)$ is a stable root, which mean that: the Jacobian matrix $\frac{\partial g}{\partial z}(x, \rho(x), 0)$ has all eigenvalues with strictly negative real part.

Assumptions 2. The reduced system Σ^0

$$\Sigma^{0} \begin{cases} \dfrac{dx}{dt} = f(x, \rho(x), 0) \\ x_{(t=0)} = x_0 \end{cases} \tag{C.1.2}$$

has a unique solution $x_0(t)$ on the segment $[0, T]$, $0 < T < +\infty$

then, with z^0 is located in the domain of attraction of the equilibrium $\rho(x^0)$ of the fast subsystem, the full system (Σ^{ϵ}) $(\epsilon << 1)$ has a unique solution $(x_{\epsilon}(t), z_{\epsilon}(t))$ on the interval $[0, T]$, and we have these following limiting relations for this solution:

$$\lim_{\epsilon \to 0^+} x_{\epsilon}(t) = x_0(t)$$

and

$$\lim_{\epsilon \to 0^+} z_{\epsilon}(t) = \rho(x_0(t)))$$

for $0 < t \leq T$.

Without adding other assumptions, in general the approximation is valid only for time in any closed interval contained in $(0, T]$. Extensions are possible for infinite time, giving therefore asymptotic Properties. For example, if the reduced system has an hyperbolic asymptotically stable equilibrium point x^*, then, if ϵ small enough, the full system also admits an hyperbolic stable equilibrium, closed to x^*. The approximation is therefore valid for infinite time, see [40].

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